Am J Physiol Gastrointest Liver Physiol 326: G712–G725, 2024.
First published April 16, 2024; doi:10.1152/ajpgi.00075.2024

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
GASTROINTESTINAL AND LIVER PHYSIOLOGY

REVIEW

Neuroimmunophysiology of the gastrointestinal tract

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Abstract

Gut physiology is the epicenter of a web of internal communication systems (i.e., neural, immune, hormonal) mediated by cell-cell contacts, soluble factors, and external influences, such as the microbiome, diet, and the physical environment. Together these provide the signals that shape enteric homeostasis and, when they go awry, lead to disease. Faced with the seemingly paradoxical tasks of nutrient uptake (digestion) and retarding pathogen invasion (host defense), the gut integrates interactions between a variety of cells and signaling molecules to keep the host nourished and protected from pathogens. When the system fails, the outcome can be acute or chronic disease, often labeled as "idiopathic" in nature (e.g., irritable bowel syndrome, inflammatory bowel disease). Here we underscore the importance of a holistic approach to gut physiology, placing an emphasis on intercellular connectedness, using enteric neuroimmunophysiology as the paradigm. The goal of this opinion piece is to acknowledge the pace of change brought to our field via single-cell and -omic methodologies and other techniques such as cell lineage tracing, transgenic animal models, methods for culturing patient tissue, and advanced imaging. We identify gaps in the field and hope to inspire and challenge colleagues to take up the mantle and advance awareness of the subtleties, intricacies, and nuances of intestinal physiology in health and disease by defining communication pathways between gut resident cells, those recruited from the circulation, and "external" influences such as the central nervous system and the gut microbiota.

enteric nervous system; epithelium; neuroimmunology; visceral afferent

INTRODUCTION

The maintenance of gastrointestinal (GI) homeostasis is a complex task, requiring the seamless integration of digestive and defensive functions of the gut (1). Neural, immune, and epithelial cell signaling systems are involved independently, but their actions are coordinated, and communication between these systems ensures that mucosal defense occurs simultaneously with digestion (2–7). The gut microbiota is not a bystander in these complex interactions but actively contributes to the neuroimmune-epithelial dialogue via a rich array of molecular signals, which vary in space and time, in a diet-dependent manner, subject to circadian control and entrainment by diurnal rhythms (8–16).

The neural control of the gut is unique in that it consists of extrinsic autonomic and primary afferent (sensory) nerves as well as a rich and complex intrinsic nervous system, the enteric nervous system (ENS) (17, 18). Communication between the intrinsic and extrinsic nerves innervating the gut, the cells of the innate and adaptive immune systems, epithelial and stromal cells ensures effective host defense functions and normal digestive function (1, 4, 19).

The first line of mucosal defense is the intestinal epithelium. Beyond the structural and secreted [e.g., HCl, mucus, defensins, secretory (s)IgA] elements of the barrier function mediated by enterocytes (20), secretory epithelial cells such as enteroendocrine cells, tuft cells, and Paneth cells are importantly involved in neuroimmune communication, transducing luminal signals to cells in the lamina propria (6, 21–25). Although neuroimmune-microbial-epithelial interactions are vital for defense, both recent and older literature support their role in digestive functions, including the regulation of motility and secretion (5, 26–28).

Together, neuroimmune-microbial-epithelial circuits ensure that digestion and defense are effectively coordinated: a break in any link of this circuit can be of pathophysiological significance. But much remains to be understood. Doing so will provide a foundation for solving an array of GI diseases, many of which lack effective therapies. Moreover, understanding the neuroimmunophysiology of the GI

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Submitted 7 March 2024 / Revised 11 April 2024 / Accepted 15 April 2024

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tract is a fundamental principle of physiology that will advance GI science in the decades to come. In this review we focus on recent literature in this field and consider knowledge gaps to stimulate new directions that will ultimately provide a more complete picture of how the gut is controlled in health and disease. Unless stated otherwise, most of the data discussed here are from murine studies.

NEUROIMMUNE COMMUNICATION

Pioneering work describing the juxtaposition of enteric nerves and mast cells (29), followed by the demonstration of bidirectional communication between these cells (30), illustrated the concept of neuroimmune interaction in the intestine. The possibility of Pavlovian conditioning of mast cell degranulation underscored the role of the brain-gut axis in this neuroimmune dialogue (31). Assessment of the nerve-mast cell unit continues to advance knowledge of connectedness among systems in the control of complex and life-saving events. For example, it was recently presented that rodent avoidance of food allergens that is associated with activation of the nucleus of the tractus solitarius in the brain stem is dependent on IgE and mast cells and occurs before evidence of enteric allergic inflammation (32). Although the authors focused on this behavioral change in mice, opportunities to define how neuroimmune-epithelial interaction affects food allergy abound. The presence of the gut bacteria can fine-tune these conditions, being recently shown to be active participants in celiac disease that had been previously thought of exclusively as a T-cell enteropathy (33). Indeed, the advent of single-cell analytics has revealed a hitherto unimaginable transcriptomic heterogeneity in mast cell populations once thought of as relatively homogeneous, with the possibility of more subtypes of a given phenotype in humans compared with mice (34).

This precedent-setting example of nerve-mast cell communication is not the exception, it is the norm: almost invariably the discovery of each new immune cell type is followed by the elucidation of its control by nerves and, reciprocally, how it can impact nerve function. Data in this area are voluminous, and we illustrate this point with recent findings on the innate lymphoid type 2 cell (ILC2), macrophages, and the surveillance/immune accessory cell, the tuft cell. ILC2 activity in inflammatory reactions can be enhanced or suppressed by enteric nerve-derived neuromedin U and calcitonin gene-related peptide (CGRP) (35–38) (Fig. 1), respectively. Vasoactive intestinal peptide (VIP) activation of ILC2s evokes the release of interleukin (IL)-5 and the recruitment of eosinophils that contributes to allergic-type responses or antihelminthic events (39) (Fig. 1). Reciprocally, the ILC2 is a potential source of acetylcholine (ACh) and so can serve as a link between luminal sensing by enteric tuft cells that release IL-25 to activate the ILC2, which in turn can propagate this "message" via ACh activation of enteric nerves (40).

Like the mast cell, the macrophage is another example of how advanced investigative methods (including mouse genetics) have allowed identification of the functional significance of cell-cell juxtaposition described years earlier. Thus, the demonstration of macrophage aggregates in the smooth muscle/myenteric plexus of *Trichinella spiralis*-infected rats (41) has been complemented by findings that during development murine macrophages in this niche refine the ENS via phagocytosis of dead neurons and pruning of synapses (42). In adulthood, these macrophages are important for the maintenance and phenotype of enteric neurons (28, 43), limit neuronal death after infection (44, 45), regulate intestinal motility (28, 46), and are essential for other aspects of gastric and intestinal homeostasis (28, 47). The description of mitochondrial transfer from macrophages to nerves in the dorsal root ganglia (DRG) of mice resulting in reduced pain signaling is intriguing (48, 49) and could have therapeutic implications in inflammatory or functional gut disease. Reciprocally, it has been shown that murine enteric glia via chemokine (C-C motif) ligand 2 (CCL2) and colony-stimulating factor 1 (CSF1) support the development of a $CD206^+$ macrophage that can exert anti-inflammatory and prohealing effects (50) (Fig. 1). The mechanisms of the cross talk between macrophages and enteric neurons that are involved in the control of motility are being elucidated (46, 51), and in the future other molecular mediators will be discovered that mediate the essential interactions between these cells.

The tuft cell exemplifies epithelial cell signaling in neuroimmune dialogue. Described 70 years ago (52), the enteric tuft cell languished in obscurity until a trilogy of studies in 2016 showed that it regulates immunity in response to infection with helminth parasites (53–55). The massive tuft cell hyperplasia driven by IL-4 after gut infection with parasitic nematodes would boost the potential of tuft cell-nerve contacts (56, 57) or "synapses," akin to the spatial interaction described for enteroendocrine cells and intrinsic and extrinsic nerves (58) (Fig. 1). Indeed, transcriptomics of mouse small intestine have indicated an immune type 2 tuft cell and a neuron-associated type 1 tuft cell (59), from which the release of ACh can affect epithelial electrolyte secretion (60). The full importance of tuft cells in surveillance of the gut lumen is far from clear, and many questions remain to be addressed. What is the role of the IL-4-independent tuft cell in gut function? What is the repertoire of external signals from the diet, the commensal bacteria, and pathogenic micro/macrobiota that these cells can detect? How is tuft cell behavior directed by endogenous signals, such as neurotransmitters? While united by a dependence on the transcription factor pou2f3 for their differentiation and characterized by Dclk1 expression, are murine small and large bowel tuft cells performing the same roles? And, of course, to what degree do murine gut tuft cells mirror human tuft cells: do the latter form a type 1 and type 2 dichotomy as described for their murine counterparts or are there more subtypes; what role, if any, does a tuft cell-nerve circuit play in, for example, idiopathic gut disease?

Broadening this concept of neuroimmunity to include the omnipresent gut bacteria, VIP has a key role in the microbial-neuroimmune control of intestinal motility (26). Bai et al. (26) showed that the gut microbiota can influence the expression of VIP in the jejunal myenteric plexus via interactions with enteric glia, and this in turn regulates cholinergic control of intestinal motility. With respect to tuft cells, bacteria-derived succinate can be sensed by GPR91 on the tuft cell, resulting in IL-25 release, with the downstream sequela mentioned above (61). Here research opportunities abound, as there seems little doubt that numerous yet to be identified

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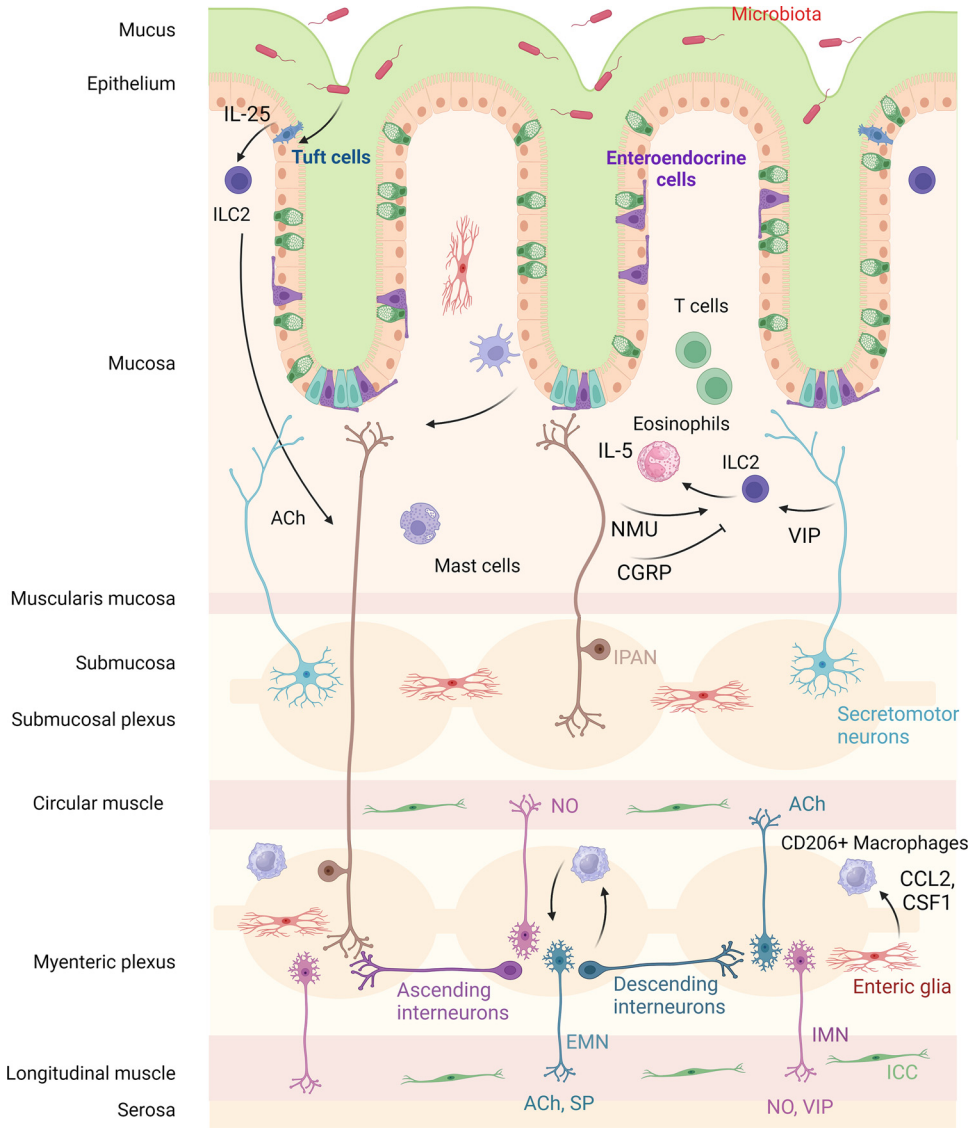

Figure 1. Enteric neuroimmune, epithelial, and enteroendocrine interactions in the gastrointestinal (GI) tract. Enteric nerves and enteric glia interact with immune, epithelial, and enteroendocrine cells to form complex circuits that promote digestion and simultaneously bolster host defense. See text for full details. ACh, acetylcholine; CCL2, chemokine (C-C motif) ligand 2; CGRP, calcitonin gene-related peptide; CSF, colony-stimulating factor; EMN, excitatory motor neuron; ICC, interstitial cells of Cajal; ILC, innate lymphoid cell; IMN, inhibitory motor neuron; IPAN, intrinsic primary afferent neuron; NO, nitric oxide; NMU, neuromedin U; SP, substance P; VIP, vasoactive intestinal peptide. Created with BioRender.com.

molecules from the microbiota will emerge as modifiers of the hosts' neuroimmune circuitry.

To summarize and speculate: The concept of enteric neuroimmunity is supported by a voluminous scientific literature that will continue to amass; for instance, the gut is the body's largest repository of eosinophils (62), and there is much to be done on how these cells affect intrinsic and extrinsic nerves of the gut and vice versa and how this functional dyad impacts surrounding stromal cells and gut physiology. Indeed, many "big questions" remain. Understanding of how the gut microbiota modifies cell-cell interactions in the gut is, with few exceptions (63), rudimentary, and the information available is heavily skewed toward the impact of bacteria, but what of viruses, fungi, and protozoan and helminth parasites? The composition of the extracellular matrix (ECM) modifies cell behavior (64) and represents a new frontier in enteric neuroimmunophysiology. The composition and stiffness of the ECM in normal and inflamed tissue are vastly different (65, 66), and awareness of this should be included in experimental design, especially in discovery in vitro analysis, where substrates composed of differing amounts of laminin, fibronectin, and collagen can be used (67). The ability to use matrices from decellularized tissues (68), control versus inflamed, to map intercellular communication could dramatically impact our understanding of enteric neuroimmunophysiology and neuroimmunopathophysiology.

ROLE OF THE EPITHELIUM IN NEUROIMMUNE REGULATION OF THE GI TRACT

The epithelium is uniquely positioned to integrate luminal signals with underlying neuroimmune regulatory components of the GI tract, including the ENS and the innate and adaptive branches of the mucosal immune system. Specific epithelial cell types respond to specific signals and interact with specific neuroimmune cells to effect targeted

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functions. For example, enteroendocrine cells detect chemical and mechanical stimuli from the lumen (69–71), relay signals to the ENS via secretion of hormones such as serotonin (5-HT), and form cell-cell contacts with afferent and efferent arms of the nervous system. Buchanan et al. (70) developed an in vivo luminal optogenetic model and demonstrated that cholecystokinin-labeled duodenal enteroendocrine cells modulate preference to luminal nutrients in the mouse gut via glutaminergic signaling to vagal afferent neurons. Tuft cells detect and respond to luminal shifts in succinate and bacterial metabolic products in the gut, equipping the cells to alert the underlying immune system of infections with protist parasites and infection with *Shigella* sp. by producing IL-25, cysteinyl leukotrienes, and prostaglandins (72, 73).

There are extensive interactions between epithelial cells, nerves, and immune cells. For example, CGRP release by nociceptors during enteric *Salmonella typhimurium* infection modulates M cell numbers, indirectly reducing the severity of the infection (74). Nociceptive neuron-derived CGRP induces goblet cell emptying in the mouse colon (75). Recent evidence suggests that ACh stimulates goblet cell antigen uptake via goblet cell antigen passages (GAPs) (76). Since GAPs are involved in developing food tolerance against common dietary antigens (77), it is worth exploring the role of neuroepithelial interactions in the development of food allergy and tolerance against common dietary antigens. Recent evidence points to immune cell regulation of epithelial absorption of nutrients; for example, γδ T cells can regulate sugar absorption by the epithelium (78).

In addition to physiological functions, neuroimmune interactions with the epithelium are closely tied to epithelial repair processes and barrier function. Several immune and inflammatory cells, while often participants in tissue damage, are also important in recovery of the epithelial barrier from inflammation (79). For example, macrophages play a dual role depending on their state of activation, contributing to inflammation via the release of proinflammatory mediators such as TNF or repair via IL-10 (79). Likewise, many immune/inflammatory mediators have a dual role in both stimulating inflammation and promoting epithelial repair, e.g., nitric oxide. Nitric oxide, made by murine macrophages and enteric glia (80–82), can cause cell damage and intestinal barrier dysfunction (83) but can also induce Wnt-induced secreted protein-1 in epithelial cells that promotes wound repair (84). Epithelial barrier function and repair are also regulated by nerves. Recent studies utilizing *Drosophila* show that cholinergic enteric neurons control epithelial intracellular Ca^{2+} via nicotinic receptors to regulate enterocyte repair (85). In mice, both direct (muscarinic receptor) and indirect (nicotinic receptor on enteric glia) cholinergic mechanisms have been found to enhance intestinal barrier function (86, 87). Neural input from extrinsic nerves can also attenuate epithelial injury. Brégeon et al. (88) showed that sacral nerve stimulation can reduce injury and enhance barrier function through changes in the distribution of tight junction proteins, a finding that has recently been translated as an adjunctive therapy for the treatment of refractory ulcerative colitis (89). The adrenergic innervation of the mucosa is also important for epithelial repair. In a mouse model of irradiation injury (90), β-adrenergic receptor signaling was found to drive IL-22 production from ILC3 cells to regulate epithelial proliferation and promote recovery (90).

Tools for Understanding the Role of the Epithelium in Neuroimmune Regulation of the GI Tract

Epithelium-targeted transgenic and knockout mouse models have significantly advanced our understanding of the roles played by the epithelium in immune and inflammatory models of GI disease (e.g., Ref. 91). New experimental approaches to understanding epithelium-nerve-immune interactions, notably intestinal organoids, single-cell RNA technologies, and the use of optogenetics (see below), will allow researchers to expand these observations to identifying the role of these interactions in both GI diseases and non-GI diseases, e.g., their role in extraintestinal inflammatory conditions such as rheumatoid arthritis (92) and neurodegenerative diseases (93), where alterations in intestinal barrier function are correlated with changes in the gut microbiota and associated with the development of these conditions.

Intestinal organoids were first described by Sato et al. in 2009 (94) and have become mainstream tools to study epithelial function in health and disease, not only in mice but in human tissue, which is relatively accessible through patient biopsies or resection specimens. Three-dimensional organoids have allowed us to learn more about epithelial cell development (84) and increased understanding of the factors that fine-tune epithelial differentiation. Two-dimensional monolayer organoids have allowed us to understand more about epithelial barrier function and repair (95, 96), without a reliance on cancer cell lines that can be abnormal in these respects. Further developing these in vitro systems into more complex coculture systems, for example culturing epithelial cells with immune cells or neurons, will provide models of primary, noncancerous human tissues derived from patients with specific diseases. Early work has shown the feasibility of these approaches, for example the coculture of epithelial cells with cultures of DRG neurons (97) or with innate immune cells (95). Related approaches involve multicell type cultures derived from induced pluripotent stem cells (iPSCs) (98). The successful iPSC-based culturing of human intestinal tissues with a functional ENS (99) is an example of how these preparations will be of enormous benefit in expanding our knowledge of how the epithelium participates in neuroimmune function.

New technologies are also facilitating the real-time spatial mapping of epithelial interactions in the context of neuroimmunophysiological regulation of the gut. Single-cell RNA sequencing is identifying new epithelial cell types that could play important roles in participation in and response to inflammation, for example, the recent description of a new repair-specific epithelial stem cell (100). In situ methodologies to spatially map gene transcription (RNAscope) and protein (multiplexed ion beam imaging) promise to help unravel the complexities of cell-cell interactions, and the role of the epithelium, in the gut neuroimmune system. Indeed, spatial profiling is already uncovering novel neuroimmune cell interactions in GI diseases including cancer (101) and inflammation (102, 103). Specifically, multiplexed spatial protein analysis identified tissue-specific neuroimmune signatures in gastroenteropancreatic neuroendocrine

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tumors, notably that duodenal gastrinomas express the proinflammatory and proneural factor IL-17B (91). Spatial profiling approaches revealed changes in gene expression of members of the endocannabinoid system (e.g., CB_2 receptor) in defined immune cell populations in murine dextran sodium sulfate-induced colitis (92) and interferon-γ-producing epithelial cells as contributors to nivolumab-associated autoimmune gastritis in humans (93).

ADVANCES IN ENTERIC NEUROBIOLOGY

Complexity in the ENS Revealed with Transcriptomic Tools

In the last 6 years, single-cell sequencing approaches have provided new insights into the molecular architecture of the ENS. Specifically, these findings reveal additional molecular subclasses of neurons that extend the preexisting chemically coded subsets of neurons defined largely by immunohistochemistry combined with lesioning and retrograde tracing (104–108). These studies reveal that much of what was done originally still holds true! That is, for the myenteric plexus, the major classes of excitatory and inhibitory motor neurons, interneurons, and intrinsic primary afferent (sensory) neurons overlap with the clustering of neuronal subtypes identified by single-cell sequencing. However, additional classes of neurons, such as new populations of interneurons and intrinsic primary afferent neurons, have been identified by single-cell sequencing whose role in the functional networks of the ENS needs to be better defined (106). These findings should prompt a reevaluation of the ENS circuitry to include not only the digestive functions that it serves to regulate but also the defensive functions of the gut. Applying this lens to the genetically defined clusters of enteric neurons will likely, if not undoubtedly, reveal specific roles for the subsets of neurons that emerge. In addition, we present three future directions that will redefine the ENS. The first is for single-cell sequencing studies to be performed in a systematic fashion on organs of the gut that have not been studied in detail, such as the stomach, duodenum, and jejunum, as this will give a more complete account of the ENS throughout the GI tract. Moreover, the conditions of the animals need to be carefully considered, since feeding status, circadian rhythms, etc. (as noted above) could influence gene expression. Second, the submucosal plexus requires an in-depth evaluation in the same way as the myenteric plexus. This is particularly crucial to understanding the full spectrum of enteric neuronal regulation in the mucosa, especially immune regulation and regulation of the epithelium and the composition of the gut microbiota. Third, transcriptomic studies should be performed in GI disorders using either well-characterized patient samples or animal models. Such studies are essential to advance the diagnosis and treatment of these disorders. Recently, a strategy utilizing single-cell RNA data combined with genomewide association studies revealed specific enteric neuron subtypes consistently linked with disorders of gut-brain interaction: functional chest pain, functional diarrhea, functional dyspepsia, functional dysphagia, and functional fecal incontinence (109). For example, substance P, neurexophilin 2, and proenkephalin neurons were positively associated with functional chest pain; nitrergic and substance P neurons were positively associated with functional diarrhea; endothelin 3 neurons were positively associated with functional dyspepsia, etc. (99). It was notable that inhibitory motor neuron subtypes, including nitrergic and neuropeptide Y neurons, were positively associated with many different disorders of gut-brain interaction, consistent with their proposed role in other GI motility disorders. This strategy also provided novel mechanistic predictions that can now be tested and, ultimately, developed into new treatments for these diseases.

Further advances will come with the use of advanced molecular techniques including spatial transcriptomics (110, 111) and integrating proteomics with single-cell transcriptomics (112). With the former, gene expression can be studied in situ, and the spatial relationships between enteric neurons, enteric glia, and other cell types in the ENS can be directly visualized and the positional context of gene expression explored in detail. For the latter, by combining transcriptomics with proteomics, one can ask whether cells are actually translating the genes that are expressed, and the protein expressions could be convenient biomarkers of disease.

Optical Tools to Probe ENS Function under Physiological Conditions

Advances in confocal microscopy and image analysis coupled with the use of mice with genetically encoded fluorescence reporter constructs (e.g., GCaMPs) have greatly advanced our understanding of enteric neural circuitry, synaptic functions, the role of extrinsic nerves in the regulation of colonic motility, and other aspects of neural control. Recent developments have made it possible to image neuronal activity in undissected preparations of the GI tract (113). With this approach, it was found that luminal distension renders the underlying myenteric circuits unresponsive to a depolarizing stimulus (113). Mechanosensitive calcium-activated potassium channel inhibition restored this responsivity, demonstrating that intestinal distension dynamically regulates the excitability of myenteric neuronal circuits. In another advance, novel analytical methods allowed the tracking of enteric neurons in the moving gut with widefield fluorescent microscopy (114). Using this approach, Barth et al. (114) found that populations of neurons exhibit distinct activity patterns in response to a variety of stimuli. One advantage of the image analysis pipeline that they developed is that it makes it possible to capture calcium dynamics from many neurons in a large field of view (114). Future studies using these types of approaches will ultimately allow for a complete understanding of enteric neural control of motility, and other functions, under completely physiological conditions, at least in the mouse.

The use of light-activated actuators and inhibitors of neuronal activity, combined with molecular genetics, offers unparalleled opportunities to probe the function of specific populations of enteric neurons, enteric glia, and other cell types in the ENS (115–118). Recently, this approach has been used to activate enterochromaffin (EC) cells (119, 120) and epithelial cells (121) and drive the intrinsic neural circuitry of

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the gut (Fig. 1). In these studies, optogenetic activation of EC cells was found to release 5-HT to drive changes in visceral sensitivity (see below) (119) and to regulate intestinal transit (120). Optogenetic activation of epithelial cells of the mouse distal, but not proximal, colon caused local contractions and increased the rate of colonic migrating motor complexes, mediated by ATP and 5-HT (121). These studies have led to new insights into epithelial signaling to the ENS, as well as neural control of motility. A recent study used this approach to stimulate β-adrenergic signaling in enteric glia, showing conclusively that sympathetic activation triggers glial reactivity and acute enteric gliosis (122). Broader adoption of these technologies will allow the field to address many key unanswered questions about the roles of specific neurons in a range of gut functions, including deciphering the reflex circuits underling host defense, which today remains to be fully elucidated.

Novel Roles for Subtypes of Enteric Glia

Remarkable advances have been made in enteric glial biology in recent years. This includes a comprehensive definition of enteric glial cell subtypes and the recent discovery of individual molecular classes of enteric glia, with one type termed a "hub cell," which expresses the mechanosensory ion channel Piezo2 (123). Selective deletion of Piezo2 from the hub cells, but not other subtypes of murine enteric glia, led to altered intestinal motility and gastric emptying (123). Similarly, distinct subtypes of enteric glia (expressing glial fibrillary acidic protein but not proteolipid protein 1) have been found to regulate intestinal stem cell niches in the intestine (124). Future studies considering the functions of specific glial subtypes will further shape this field.

Although the role of enteric glia in enteric neurotransmission has been known for some time, how this is integrated in functional neural circuits remains to be fully understood. Gulbransen's group have recently shown that subsets of enteric glia are recruited by polarized (ascending, descending, and circumferential) neural pathways and that glial activation produces effects that are specific to each neural pathway (125). These findings demonstrate that enteric glia have the potential to function in concert with enteric neurons as neuro-glial units in the ENS circuitry. Whether they do so in other circuits has to be determined.

Recent studies have highlighted the significance of interactions between enteric glia and immune cells in the gut for shaping pathophysiological responses. Thus, Schneider et al. (126) showed that glucocorticoids, released during stress, activate enteric glia to promote inflammation in the gut via the release of CSF1 that triggers enteric macrophages to release tumor necrosis factor (Fig. 1). These findings illustrate how important enteric glia are in the physiology and pathophysiology of neuroimmune functions in the gut.

NEUROIMMUNE-MICROBIOTA INTERACTIONS IN THE REGULATION OF VISCERAL SENSITIVITY

The GI tract is innervated by two extrinsic sensory pathways: vagal and spinal afferents. These sensory pathways have distinct origins and encode different stimuli (127–130). Vagal afferents have their soma in the nodose and jugular ganglia, whereas spinal afferents originate from the DRG (127). Although some evidence suggests that vagal afferents can respond to nociceptive stimuli (131), visceral pain is transmitted via spinal afferents, which project to the dorsal horn of the spinal cord (127). Vagal afferents are an essential conduit of the gut-brain-microbiota axis, responding to neural, enteroendocrine, immune, and microbial signals conveying the "state of the gut" to the brain (128, 132–134). The brain in turn initiates and coordinates appropriate feedback including neural, behavioral, and endocrine responses that control digestive and defensive functions of the gut.

Nociceptive mediators originating from epithelial cells, immune cells, and nerves, as a result of injury, infection or inflammation, or metabolites from the gut microbiota, impact pain circuits in the periphery (GI tract) and centrally (spinal cord and brain), contributing to visceral hypersensitivity (VHS) (135) (Fig. 2). The role of enteric glia in VHS was previously unclear, but recent research by Grubišić et al. (136) has emphasized the importance of communication between enteric glia and immune cells in modulating VHS. Morales-Soto et al. (137) found that enteric glia promote VHS by the production of prostaglandin E_2 during inflammation by signaling to extrinsic primary afferent nerves whose terminals surround enteric glia in the myenteric plexus (Fig. 2). These new findings highlight an interesting neural-immune linkage not previously recognized.

Neuroimmune signaling in the gut is not only nociceptive but can also be antinociceptive. In inflammatory conditions, local release of opioids by leukocytes at the inflammatory site inhibits nociceptor firing, inducing analgesia. For example, in chronic intestinal Th1/Th17-associated inflammation, enkephalins released by colitogenic $CD4^+$ T lymphocytes alleviate inflammation-induced visceral pain (138) (Fig. 2). The endocannabinoid system may be playing similar roles, but these have yet to be fully elucidated (139).

The epithelium acts as a crucial interface between luminal contents and spinal sensory afferents. In the colon, the EC cells produce and release 90% of the body's 5-HT. A recent study revealed that 5-HT released from EC cells may initiate visceral pain and anxiety associated with conditions like irritable bowel syndrome (IBS) and other inflammatory GI disorders (119). Additionally, a compromised intestinal barrier can lead to pain due to the infiltration of sensitizing agents from the diet and/or microbiota. Aguilera-Lizarraga et al. (140) uncovered a novel peripheral mechanism underlying food-induced abdominal pain. Bacterial infection and toxins can trigger an immune response, leading to the production of dietary antigen-specific IgE antibodies in mice, primarily within the intestine. Subsequent oral ingestion of the respective dietary antigen results in increased visceral pain through an IgE- and mast cell-dependent mechanism (140).

Early indicators of the role the microbiota plays in visceral hypersensitivity arose from studies with antibiotics. Thus, mice treated with antibiotics had the expected altered gut microbiota composition and also increased inflammatory markers and visceral hypersensitivity (141). The gut microbiota can stimulate nociceptors, through the production of metabolites such as short-chain fatty acids (142), toxins

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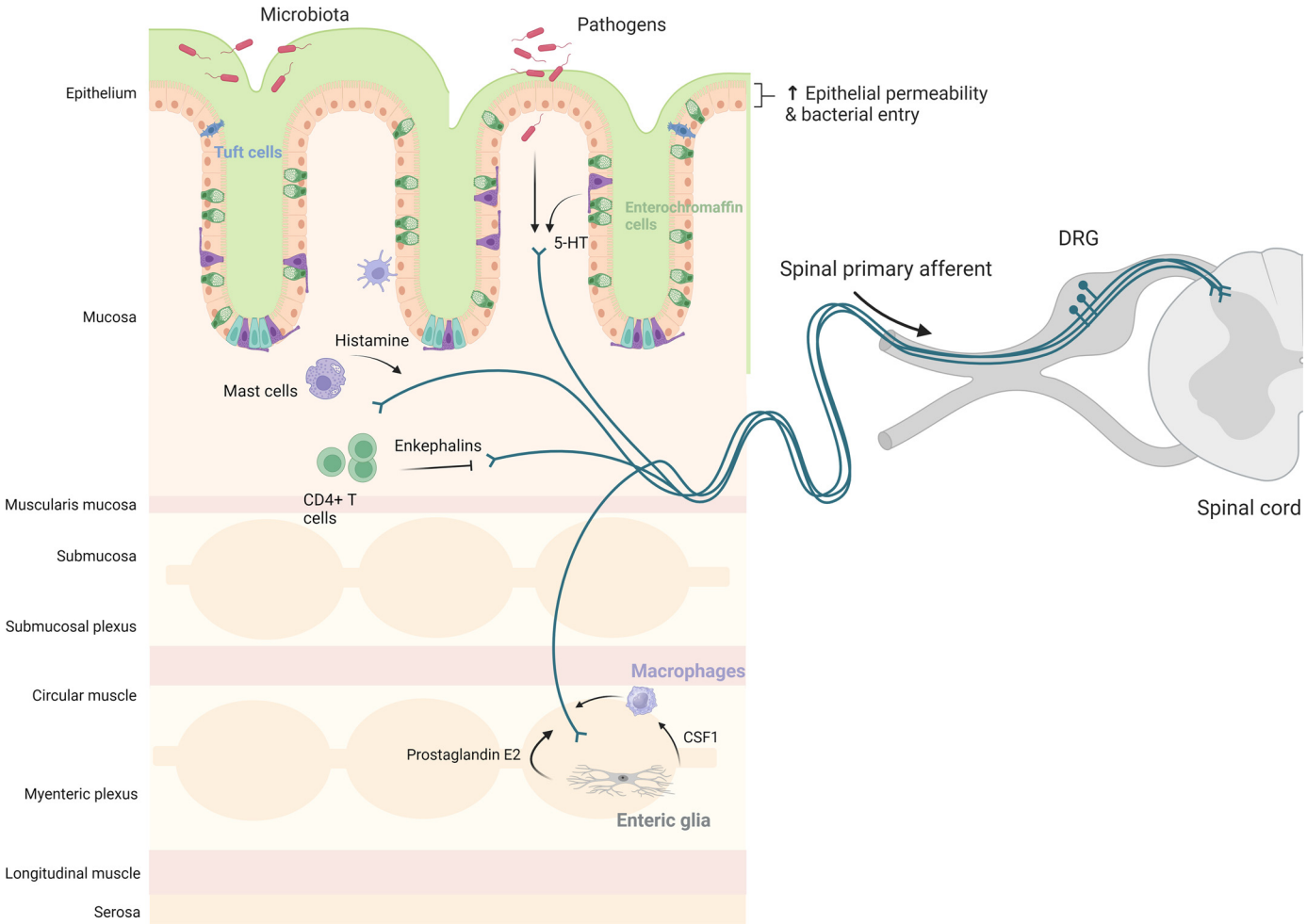

Figure 2. Neuroimmune interactions in the gastrointestinal (GI) tract regulate visceral sensitivity. The activity in spinal primary afferent nerves is regulated by epithelial, enteroendocrine, immune, and enteric glial interactions. Spinal primary afferents are activated in conditions of inflammation or infection, and this can be sustained by sensitization and via alterations in the composition of the gut microbiota. See text for full details. CSF, colony-stimulating factor; DRG, dorsal root ganglia. Created with BioRender.com.

(143), the neurotransmitter catecholamines, histamine, and GABA (144). The gut microbiota can also indirectly affect pain signaling via the fermentation of dietary fibers. A recent study demonstrated that a diet high in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) led to increased urinary histamine in a subset of patients with IBS (145). Furthermore, when germ-free mice were colonized with fecal samples from high histamine-producing patients, they exhibited increased pain to colorectal distension. This effect was inhibited by both histamine-1 receptor and histamine-4 receptor antagonism as well as by a low-fermentable diet. Remarkably, the source of histamine production was found to be both mast cell and bacteria derived (146). Overall, these findings suggest that microbiota can alter colonic afferent sensitivity via their roles in barrier function, immune responses, as well as metabolite-epithelial-neuronal, metabolite-neuronal, or bacterial-neuronal interactions. Microbial dysbiosis likely changes the balance of these mechanisms, potentially resulting in persistent sensitization of spinal afferents via a variety of mechanisms that require elucidation.

Gut microbial dysbiosis also results in changes in cognitive function and behavior, particularly in the critical neonatal period. Mice exposed to broad-spectrum antibiotics as neonates demonstrate impaired myelin formation, impaired cognition, and increased anxiety-like behaviors as adults, effects that were reversed by butyrate (147). Neonatal enteropathogenic *Escherichia coli* infection caused impaired barrier function, increased hippocampal cytokine production, and microglial activation, which was coupled with increased memory recognition deficits in adults (148). Together, these data suggest that the gut-brain-microbiota axis is critical in the pathogenesis of visceral pain and modulates behavior, underscoring the putative contribution of this axis to neurodevelopmental disease (149).

Future directions in the gut-brain-microbiome axis include the use of novel techniques to evaluate mechanistic interactions between gut microbes, epithelial, immune, and neural cells (see CHEMOGENETICS: AN UPCOMING MODEL and HUMANIZED GNOTOBIOTIC MOUSE MODEL TO STUDY PAIN), as well as evaluating what role biological sex plays in these

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complex interactions. Women are disproportionately affected by chronic pain disorders, both IBS and inflammatory bowel disease (IBD) in remission (150–152). Several exciting studies have demonstrated that there is a complex interplay between sex hormones, the gut microbiome, and visceral hypersensitivity. The visceral motor response to colorectal distension is known to vary across the estrous cycle; this effect is abolished in a germ-free environment (153). Germ-free animals have increased visceral responses to colorectal distension, and in female mice this effect appears to be driven in part by increased CGRP expression (154). Mucosal afferent nerve-EC cell circuits are tonically active in female, but not male, mice and are not responsive to stimulation by the microbial metabolite isovalerate (119). Further studies evaluating biological sex as a key variable are necessary to understand the pathophysiology of chronic visceral pain.

CHEMOGENETICS: AN UPCOMING MODEL

Over the past two decades, optogenetic and chemogenetic techniques have revolutionized the field of neuroscience and other disciplines (155, 156). These approaches enable the reversible, cell type-specific control of cellular signaling and electrical activity (157, 158). Among these technologies, the chemogenetic platform known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) activated by a synthetic, pharmacologically inert ligand (155) has been demonstrated to be exceptionally valuable for investigating cellular signaling associated with the gut-brain axis, including neurons (159), glia (137, 160), and enterochromaffin cells (119). Although DREADDs lack the precise temporal control afforded by optical technologies (156), they may prove to be well suited for translational applications, which typically occur via chronic modulation of G protein-coupled receptor signaling.

HUMANIZED GNOTOBIOTIC MOUSE MODELS TO STUDY PAIN

Considerable evidence highlights the critical role of the gut microbiome in the gut-brain axis. The potential therapeutic application is gaining increased attention. However, key questions such as the identification of suitable microorganisms and the selection of relevant preclinical models that can faithfully replicate the human microbiota remain largely unanswered. The establishment of a humanized gnotobiotic mouse model through human fecal microbiota transplantation into germ-free mice provides an innovative and powerful tool that can more easily allow for translation of preclinical findings to patients (146). However, many considerations are required in designing such a model, as numerous elements, ranging from the factors pertaining to human donors to the mouse genetic background, affect how microorganisms colonize the mouse gut. Thus, it is critical to match the murine context to that of human donors to provide a continuous and faithful progression of human microbiota in mice.

THE FUTURE IS BRIGHT

Single-cell analytics is identifying, and will continue to identify, stromal, immune, epithelial, and neural cell subtypes, the functions of which need to be addressed. The rarity of some of these cell types does not undermine their importance but adds technical challenges for functional analysis. The ability to image in real time interactions between lineage-labeled immune cells and nerves, and subtypes thereof, will be illuminating. Finally, what role, if any, is there for in silico analyses in defining neuroimmunophysiological interactions? The availability of single-cell database repositories combined with computational bioinformatics can inform on putative functional cell phenotypes, and pathway analysis can generate testable hypotheses, approaches that may be particularly useful for human studies where biological samples can be challenging to obtain or in the context of rare-disease phenotypes. We hope we have intrigued the reader by drawing attention to some tantalizing findings in the gut that have important parallels in other mucosal tissues (161) and that can open up new areas of research for the aspiring enteric neuroimmunophysiologist. Many key questions remain unanswered including:

1. How do polarized enteric neural circuits regulate the neuroimmune signaling throughout the gut?
2. What bacteria-derived metabolites directly or indirectly affect enteric neural circuitry and brain function?
3. Which cells mediate indirect microbial interactions with the enteric innervation?
4. Are there sex differences in neuroimmune circuitry throughout the gut or specific regions that predispose or protect against disease?
5. How is neuroimmune control of digestion and mucosal defense affected throughout development, notably, the neonatal period, adolescence, and aging?
6. How does redundancy in neuroimmune circuitry in the gut affect disease pathogenesis? How do we identify targets with translational relevance?
7. How do environmental contaminants/pollutants and food additives affect enteric neuroimmune circuitry and the propensity to develop inflammatory disease and/or vulnerability to infectious disease?
8. How does epigenetic imprinting of enteric neurons and glia impact intestinal homeostasis?
9. Can variability in neuroimmune responses be targeted to enable personalized treatments in immune and inflammatory diseases of the gut?

Of course the big challenge for the curious is the discovery of the "unknown unknowns" that impact gut physiology and pathophysiology, discoveries that the aforementioned technologies place within our grasp.

ACKNOWLEDGMENTS

We thank Eve Higgins for assistance with the graphical abstract. We highlight what we believe are important recent findings in the area, while recognizing that many outstanding studies will have been missed. We apologize in advance to any colleagues whose work has been omitted to maintain the length of this paper. This paper arose because of a recent conference that we organized

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(https://snyder.ucalgary.ca/neuroimmunophysiology-conference). We are grateful to the speakers, participants, and sponsors of the conference, at which many of the ideas for this article were conceived.

GRANTS

Original work in the authors' laboratories is supported by grants from the Canadian Institutes of Health Research, the Weston Foundation Microbiome Initiative, Crohn's and Colitis Canada, and the Leona M. and Harry B. Helmsley Charitable Trust.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.M.M., M.D., S.R., W.K.M., Y.N., and K.A.S. conceived and designed research; M.D. and K.A.S. prepared figures; D.M.M., M.D., S.R., W.K.M., Y.N., and K.A.S. drafted manuscript; D.M.M., M.D., S.R., W.K.M., Y.N., and K.A.S. edited and revised manuscript; D.M.M., M.D., S.R., W.K.M., Y.N., and K.A.S. approved final version of manuscript.

REFERENCES

1. **Sharkey KA**, **Beck PL**, **McKay DM.** Neuroimmunophysiology of the gut: advances and emerging concepts focusing on the epithelium. *Nat Rev Gastroenterol Hepatol* 15: 765–784, 2018. doi:10.1038/s41575-018-0051-4.
2. **Neunlist M**, **Van Landeghem L**, **Mahé MM**, **Derkinderen P**, **Des Varannes SB**, **Rolli-Derkinderen M.** The digestive neuronal-glial-epithelial unit: a new actor in gut health and disease. *Nat Rev Gastroenterol Hepatol* 10: 90–100, 2013. doi:10.1038/nrgastro.2012.221.
3. **Chu C**, **Artis D**, **Chiu IM.** Neuro-immune interactions in the tissues. *Immunity* 52: 464–474, 2020. doi:10.1016/j.immuni.2020.02.017.
4. **Klein Wolterink RG**, **Wu GS**, **Chiu IM**, **Veiga-Fernandes H.** Neuroimmune interactions in peripheral organs. *Annu Rev Neurosci* 45: 339–360, 2022. doi:10.1146/annurev-neuro-111020-105359.
5. **Fung C**, **Vanden Berghe P.** Functional circuits and signal processing in the enteric nervous system. *Cell Mol Life Sci* 77: 4505–4522, 2020. doi:10.1007/s00018-020-03543-6.
6. **Kaelberer MM**, **Rupprecht LE**, **Liu WW**, **Weng P**, **Bohórquez DV.** Neuropod cells: the emerging biology of gut-brain sensory transduction. *Annu Rev Neurosci* 43: 337–353, 2020. doi:10.1146/annurev-neuro-091619-022657.
7. **Seguella L**, **Gulbransen BD.** Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. *Nat Rev Gastroenterol Hepatol* 18: 571–587, 2021. doi:10.1038/s41575-021-00423-7.
8. **Godinho-Silva C**, **Domingues RG**, **Rendas M**, **Raposo B**, **Ribeiro H**, **da Silva JA**, **Vieira A**, **Costa RM**, **Barbosa-Morais NL**, **Carvalho T**, **Veiga-Fernandes H.** Light-entrained and brain-tuned circadian circuits regulate ILC3s and gut homeostasis. *Nature* 574: 254–258, 2019. doi:10.1038/s41586-019-1579-3.
9. **Heddes M**, **Altaha B**, **Niu Y**, **Reitmeier S**, **Kleigrewe K**, **Haller D**, **Kiessling S.** The intestinal clock drives the microbiome to maintain gastrointestinal homeostasis. *Nat Commun* 13: 6068, 2022. doi:10.1038/s41467-022-33609-x.
10. **Brooks JF 2nd**, **Behrendt CL**, **Ruhn KA**, **Lee S**, **Raj P**, **Takahashi JS**, **Hooper LV.** The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *Cell* 184: 4154–4167.e12, 2021. doi:10.1016/j.cell.2021.07.001.
11. **Frazier K**, **Kambal A**, **Zale EA**, **Pierre JF**, **Hubert N**, **Miyoshi S**, **Miyoshi J**, **Ringus DL**, **Harris D**, **Yang K**, **Carroll K**, **Hermanson JB**, **Chlystek JS**, **Overmyer KA**, **Cham CM**, **Musch MW**, **Coon JJ**, **Chang EB**, **Leone VA.** High-fat diet disrupts REG3gamma and gut microbial rhythms promoting metabolic dysfunction. *Cell Host Microbe* 30: 809–823.e6, 2022. doi:10.1016/j.chom.2022.03.030.
12. **Kuang Z**, **Wang Y**, **Li Y**, **Ye C**, **Ruhn KA**, **Behrendt CL**, **Olson EN**, **Hooper LV.** The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. *Science* 365: 1428–1434, 2019. doi:10.1126/science.aaw3134.
13. **Leone V**, **Gibbons SM**, **Martinez K**, **Hutchison AL**, **Huang EY**, **Cham CM**, **Pierre JF**, **Heneghan AF**, **Nadimpalli A**, **Hubert N**, **Zale E**, **Wang Y**, **Huang Y**, **Theriault B**, **Dinner AR**, **Musch MW**, **Kudsk KA**, **Prendergast BJ**, **Gilbert JA**, **Chang EB.** Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 17: 681–689, 2015. doi:10.1016/j.chom.2015.03.006.
14. **Thaiss CA**, **Levy M**, **Korem T**, **Dohnalová L**, **Shapiro H**, **Jaitin DA**, **David E**, **Winter DR**, **Gury-BenAri M**, **Tatirovsky E**, **Tuganbaev T**, **Federici S**, **Zmora N**, **Zeevi D**, **Dori-Bachash M**, **Pevsner-Fischer M**, **Kartvelishvily E**, **Brandis A**, **Harmelin A**, **Shibolet O**, **Halpern Z**, **Honda K**, **Amit I**, **Segal E**, **Elinav E.** Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167: 1495–1510.e12, 2016. doi:10.1016/j.cell.2016.11.003.
15. **Wang Y**, **Kuang Z**, **Yu X**, **Ruhn KA**, **Kubo M**, **Hooper LV.** The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science* 357: 912–916, 2017. doi:10.1126/science.aan0677.
16. **Tuganbaev T**, **Mor U**, **Bashiardes S**, **Liwinski T**, **Nobs SP**, **Leshem A**, **Dori-Bachash M**, **Thaiss CA**, **Pinker EY**, **Ratiner K**, **Adlung L**, **Federici S**, **Kleimeyer C**, **Moresi C**, **Yamada T**, **Cohen Y**, **Zhang X**, **Massalha H**, **Massasa E**, **Kuperman Y**, **Koni PA**, **Harmelin A**, **Gao N**, **Itzkovitz S**, **Honda K**, **Shapiro H**, **Elinav E.** Diet diurnally regulates small intestinal microbiome-epithelial-immune homeostasis and enteritis. *Cell* 182: 1441–1459.e21, 2020. doi:10.1016/j.cell.2020.08.027.
17. **Sharkey KA**, **Mawe GM.** The enteric nervous system. *Physiol Rev* 103: 1487–1564, 2023. doi:10.1152/physrev.00018.2022.
18. **Populin L**, **Stebbing MJ**, **Furness JB.** Neuronal regulation of the gut immune system and neuromodulation for treating inflammatory bowel disease. *FASEB Bioadv* 3: 953–966, 2021. doi:10.1096/fba.2021-00070.
19. **Ramanan D**, **Cadwell K.** Intrinsic defense mechanisms of the intestinal epithelium. *Cell Host Microbe* 19: 434–441, 2016. doi:10.1016/j.chom.2016.03.003.
20. **Horowitz A**, **Chanez-Paredes SD**, **Haest X**, **Turner JR.** Paracellular permeability and tight junction regulation in gut health and disease. *Nat Rev Gastroenterol Hepatol* 20: 417–432, 2023. doi:10.1038/s41575-023-00766-3.
21. **Bas J**, **Jay P**, **Gerbe F.** Intestinal tuft cells: sentinels, what else? *Semin Cell Dev Biol* 150-151: 35–42, 2023. doi:10.1016/j.semcdb.2023.02.012.
22. **Billipp TE**, **Nadjsombati MS**, **von Moltke J.** Tuning tuft cells: new ligands and effector functions reveal tissue-specific function. *Curr Opin Immunol* 68: 98–106, 2021. doi:10.1016/j.coi.2020.09.006.
23. **Lueschow SR**, **McElroy SJ.** The Paneth cell: the curator and defender of the immature small intestine. *Front Immunol* 11: 587, 2020. doi:10.3389/fimmu.2020.00587.
24. **Mercado-Perez A**, **Beyder A.** Gut feelings: mechanosensing in the gastrointestinal tract. *Nat Rev Gastroenterol Hepatol* 19: 283–296, 2022. doi:10.1038/s41575-021-00561-y.
25. **Worthington JJ**, **Reimann F**, **Gribble FM.** Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. *Mucosal Immunol* 11: 3–20, 2018. doi:10.1038/mi.2017.73.
26. **Bai X**, **De Palma G**, **Boschetti E**, **Nishiharo Y**, **Lu J**, **Shimbori C**, **Costanzini A**, **Saqib Z**, **Kraimi N**, **Sidani S**, **Hapfelmeier S**, **Macpherson AJ**, **Verdu EF**, **De Giorgio R**, **Collins SM**, **Bercik P.** Vasoactive intestinal polypeptide plays a key role in the microbial-neuroimmune control of intestinal motility. *Cell Mol Gastroenterol Hepatol* 17: 383–398, 2024. doi:10.1016/j.jcmgh.2023.11.012.
27. **Cooke HJ.** Neuroimmune signaling in regulation of intestinal ion transport. *Am J Physiol Gastrointest Liver Physiol* 266: G167–G178, 1994. doi:10.1152/ajpgi.1994.266.2.G167.
28. **De Schepper S**, **Verheijden S**, **Aguilera-Lizarraga J**, **Viola MF**, **Boesmans W**, **Stakenborg N**, **Voytyuk I**, **Schmidt I**, **Boeckx B**, **Dierckx de Casterlé I**, **Baekelandt V**, **Gonzalez Dominguez E**, **Mack M**, **Depoortere I**, **De Strooper B**, **Sprangers B**, **Himmelreich U**, **Soenen S**, **Guilliams M**, **Vanden Berghe P**, **Jones E**, **Lambrechts D**,

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Boeckxstaens G. Self-maintaining gut macrophages are essential for intestinal homeostasis. *Cell* 175: 400–415.e13, 2018. doi:10.1016/j.cell.2018.07.048.

29. **Stead RH**, **Tomioka M**, **Quinonez G**, **Simon GT**, **Felten SY**, **Bienenstock J.** Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 84: 2975–2979, 1987. doi:10.1073/pnas.84.9.2975.
30. **Suzuki R**, **Furuno T**, **McKay DM**, **Wolvers D**, **Teshima R**, **Nakanishi M**, **Bienenstock J.** Direct neurite-mast cell communication in vitro occurs via the neuropeptide substance P. *J immunol* 163: 2410–2415, 1999. doi:10.4049/jimmunol.163.5.2410.
31. **MacQueen G**, **Marshall J**, **Perdue M**, **Siegel S**, **Bienenstock J.** Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. *Science* 243: 83–85, 1989. doi:10.1126/science.2911721.
32. **Florsheim EB**, **Bachtel ND**, **Cullen JL**, **Lima BG**, **Godazgar M**, **Carvalho F**, **Chatain CP**, **Zimmer MR**, **Zhang C**, **Gautier G**, **Launay P**, **Wang A**, **Dietrich MO**, **Medzhitov R.** Immune sensing of food allergens promotes avoidance behaviour. *Nature* 620: 643–650, 2023. doi:10.1038/s41586-023-06362-4.
33. **Caminero A**, **McCarville JL**, **Galipeau HJ**, **Deraison C**, **Bernier SP**, **Constante M**, **Rolland C**, **Meisel M**, **Murray JA**, **Yu XB**, **Alaedini A**, **Coombes BK**, **Bercik P**, **Southward CM**, **Ruf W**, **Jabri B**, **Chirdo FG**, **Casqueiro J**, **Surette MG**, **Vergnolle N**, **Verdu EF.** Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2. *Nat Commun* 10: 1198, 2019. doi:10.1038/s41467-019-09037-9.
34. **Tauber M**, **Basso L**, **Martin J**, **Bostan L**, **Pinto MM**, **Thierry GR**, **Houmadi R**, **Serhan N**, **Loste A**, **Blériot C**, **Kamphuis JB**, **Grujic M**, **Kjellén L**, **Pejler G**, **Paul C**, **Dong X**, **Galli SJ**, **Reber LL**, **Ginhoux F**, **Bajenoff M**, **Gentek R**, **Gaudenzio N.** Landscape of mast cell populations across organs in mice and humans. *J Exp Med* 220: e20230570, 2023 [Erratum in J Exp Med 221: e202305700112024c, 2024]. doi:10.1084/jem.20230570.
35. **Nagashima H**, **Mahlakõiv T**, **Shih HY**, **Davis FP**, **Meylan F**, **Huang Y**, **Harrison OJ**, **Yao C**, **Mikami Y**, **Urban JF Jr**, **Caron KM**, **Belkaid Y**, **Kanno Y**, **Artis D**, **O'Shea JJ.** Neuropeptide CGRP Limits group 2 innate lymphoid cell responses and constrains type 2 inflammation. *Immunity* 51: 682–695.e6, 2019. doi:10.1016/j.immuni.2019.06.009.
36. **Cardoso V**, **Chesné J**, **Ribeiro H**, **García-Cassani B**, **Carvalho T**, **Bouchery T**, **Shah K**, **Barbosa-Morais NL**, **Harris N**, **Veiga-Fernandes H.** Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* 549: 277–281, 2017. doi:10.1038/nature23469.
37. **Tsou AM**, **Yano H**, **Parkhurst CN**, **Mahlakõiv T**, **Chu C**, **Zhang W**, **He Z**, **Jarick KJ**, **Zhong C**, **Putzel GG**, **Hatazaki M**, **Consortium J**, **Lorenz IC**, **Andrew D**, **Balderes P**, **Klose CS**, **Lira SA**, **Artis D**; **JRI IBD Live Cell Bank Consortium.** Neuropeptide regulation of non-redundant ILC2 responses at barrier surfaces. *Nature* 611: 787–793, 2022. doi:10.1038/s41586-022-05297-6.
38. **Xu H**, **Ding J**, **Porter CBM**, **Wallrapp A**, **Tabaka M**, **Ma S**, **Fu S**, **Guo X**, **Riesenfeld SJ**, **Su C**, **Dionne D**, **Nguyen LT**, **Lefkovith A**, **Ashenberg O**, **Burkett PR**, **Shi HN**, **Rozenblatt-Rosen O**, **Graham DB**, **Kuchroo VK**, **Regev A**, **Xavier RJ.** Transcriptional atlas of intestinal immune cells reveals that neuropeptide alpha-CGRP modulates group 2 innate lymphoid cell responses. *Immunity* 51: 696–708.e9, 2019. doi:10.1016/j.immuni.2019.09.004.
39. **Nussbaum JC**, **Van Dyken SJ**, **von Moltke J**, **Cheng LE**, **Mohapatra A**, **Molofsky AB**, **Thornton EE**, **Krummel MF**, **Chawla A**, **Liang HE**, **Locksley RM.** Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 502: 245–248, 2013. doi:10.1038/nature12526.
40. **Chu C**, **Parkhurst CN**, **Zhang W**, **Zhou L**, **Yano H**, **Arifuzzaman M**, **Artis D.** The ChAT-acetylcholine pathway promotes group 2 innate lymphoid cell responses and anti-helminth immunity. *Sci Immunol* 6: eabe3218, 2021. doi:10.1126/sciimmunol.abe3218.
41. **Berezin I**, **Huizinga JD**, **Collins SM.** Structural relationships between immune cells and longitudinal muscle during a *Trichinella spiralis* infection in the rat intestine. *Neurogastroenterol Motil* 11: 179–192, 1999. doi:10.1046/j.1365-2982.1999.00152.x.
42. **Viola MF**, **Chavero-Pieres M**, **Modave E**, **Delfini M**, **Stakenborg N**, **Estévez MC**, **Fabre N**, **Appeltans I**, **Martens T**, **Vandereyken K**, **Theobald H**, **Van Herck J**, **Petry P**, **Verheijden S**, **De Schepper S**, **Sifrim A**, **Liu Z**, **Ginhoux F**, **Azhar M**, **Schlitzer A**, **Matteoli G**, **Kierdorf K**, **Prinz M**, **Vanden Berghe P**, **Voet T**, **Boeckxstaens G.** Dedicated macrophages organize and maintain the enteric nervous system. *Nature* 618: 818–826, 2023. doi:10.1038/s41586-023-06200-7.
43. **Cipriani G**, **Terhaar ML**, **Eisenman ST**, **Ji S**, **Linden DR**, **Wright AM**, **Sha L**, **Ordog T**, **Szurszewski JH**, **Gibbons SJ**, **Farrugia G.** Muscularis propria macrophages alter the proportion of nitrergic but not cholinergic gastric myenteric neurons. *Cell Mol Gastroenterol Hepatol* 7: 689–691.e4, 2019. doi:10.1016/j.jcmgh.2019.01.005.
44. **Ahrends T**, **Aydin B**, **Matheis F**, **Classon CH**, **Marchildon F**, **Furtado GC**, **Lira SA**, **Mucida D.** Enteric pathogens induce tissue tolerance and prevent neuronal loss from subsequent infections. *Cell* 184: 5715–5727.e2, 2021. doi:10.1016/j.cell.2021.10.004.
45. **Matheis F**, **Muller PA**, **Graves CL**, **Gabanyi I**, **Kerner ZJ**, **Costa-Borges D**, **Ahrends T**, **Rosenstiel P**, **Mucida D.** Adrenergic signaling in muscularis macrophages limits infection-induced neuronal loss. *Cell* 180: 64–78.e16, 2020. doi:10.1016/j.cell.2019.12.002.
46. **Muller PA**, **Koscsó B**, **Rajani GM**, **Stevanovic K**, **Berres ML**, **Hashimoto D**, **Mortha A**, **Leboeuf M**, **Li XM**, **Mucida D**, **Stanley ER**, **Dahan S**, **Margolis KG**, **Gershon MD**, **Merad M**, **Bogunovic M.** Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* 158: 300–313, 2014 [Erratum in Cell 158: 1210, 2014]. doi:10.1016/j.cell.2014.04.050.
47. **Cipriani G**, **Gibbons SJ**, **Miller KE**, **Yang DS**, **Terhaar ML**, **Eisenman ST**, **Ördög T**, **Linden DR**, **Gajdos GB**, **Szurszewski JH**, **Farrugia G.** Change in populations of macrophages promotes development of delayed gastric emptying in mice. *Gastroenterology* 154: 2122–2136.e12, 2018. doi:10.1053/j.gastro.2018.02.027.
48. **van der Vlist M**, **Raoof R**, **Willemen H**, **Prado J**, **Versteeg S**, **Martin Gil C**, **Vos M**, **Lokhorst RE**, **Pasterkamp RJ**, **Kojima T**, **Karasuyama H**, **Khoury-Hanold W**, **Meyaard L**, **Eijkelkamp N.** Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. *Neuron* 110: 613–626.e9, 2022. doi:10.1016/j.neuron.2021.11.020.
49. **Geng Z**, **Guan S**, **Wang S**, **Yu Z**, **Liu T**, **Du S**, **Zhu C.** Intercellular mitochondrial transfer in the brain, a new perspective for targeted treatment of central nervous system diseases. *CNS Neurosci Ther* 29: 3121–3135, 2023. doi:10.1111/cns.14344.
50. **Stakenborg M**, **Abdurahiman S**, **De Simone V**, **Goverse G**, **Stakenborg N**, **van Baarle L**, **Wu Q**, **Pirottin D**, **Kim JS**, **Chappell-Maor L**, **Pintelon I**, **Thys S**, **Pollenus E**, **Boon L**, **Van den Steen P**, **Hao M**, **Van Ginderachter JA**, **Boeckxstaens GE**, **Timmermans JP**, **Jung S**, **Marichal T**, **Ibiza S**, **Matteoli G.** Enteric glial cells favor accumulation of anti-inflammatory macrophages during the resolution of muscularis inflammation. *Mucosal Immunol* 15: 1296–1308, 2022. doi:10.1038/s41385-022-00563-2.
51. **Pendse M**, **De Selle H**, **Vo N**, **Quinn G**, **Dende C**, **Li Y**, **Salinas CN**, **Srinivasan T**, **Propheter DC**, **Crofts AA**, **Koo E**, **Hassell B**, **Ruhn KA**, **Raj P**, **Obata Y**, **Hooper LV.** Macrophages regulate gastrointestinal motility through complement component 1q. *eLife* 12: e78558, 2023. doi:10.7554/eLife.78558.
52. **Järvi O**, **Keyriläinen O.** On the cellular structures of the epithelial invasions of the glandular stomach of mice caused by intramural application of 20-methylcholantren. *Acta Pathol Microbiol Scand* 38: 72–73, 1956. doi:10.1111/j.1600-0463.1956.tb06739.x.
53. **Gerbe F**, **Sidot E**, **Smyth DJ**, **Ohmoto M**, **Matsumoto I**, **Dardalhon V**, **Cesses P**, **Garnier L**, **Pouzolles M**, **Brulin B**, **Bruschi M**, **Harcus Y**, **Zimmermann VS**, **Taylor N**, **Maizels RM**, **Jay P.** Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* 529: 226–230, 2016. doi:10.1038/nature16527.
54. **Howitt MR**, **Lavoie S**, **Michaud M**, **Blum AM**, **Tran SV**, **Weinstock JV**, **Gallini CA**, **Redding K**, **Margolskee RF**, **Osborne LC**, **Artis D**, **Garrett WS.** Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351: 1329–1333, 2016. doi:10.1126/science.aaf1648.
55. **von Moltke J**, **Ji M**, **Liang HE**, **Locksley RM.** Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 529: 221–225, 2016. doi:10.1038/nature16161.
56. **Cheng X**, **Voss U**, **Ekblad E.** Tuft cells: distribution and connections with nerves and endocrine cells in mouse intestine. *Exp Cell Res* 369: 105–111, 2018. doi:10.1016/j.yexcr.2018.05.011.
57. **O'Leary CE**, **Schneider C**, **Locksley RM.** Tuft cells—systemically dispersed sensory epithelia integrating immune and neural circuitry. *Annu Rev Immunol* 37: 47–72, 2019. doi:10.1146/annurev-immunol-042718-041505.

Downloaded from journals.physiology.org/journal/ajpgi (172.232.036.207) on November 21, 2025.

58. **Bohórquez DV**, **Shahid RA**, **Erdmann A**, **Kreger AM**, **Wang Y**, **Calakos N**, **Wang F**, **Liddle RA.** Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest* 125: 782–786, 2015. doi:10.1172/JCI78361.
59. **Haber AL**, **Biton M**, **Rogel N**, **Herbst RH**, **Shekhar K**, **Smillie C**, **Burgin G**, **Delorey TM**, **Howitt MR**, **Katz Y**, **Tirosh I**, **Beyaz S**, **Dionne D**, **Zhang M**, **Raychowdhury R**, **Garrett WS**, **Rozenblatt-Rosen O**, **Shi HN**, **Yilmaz O**, **Xavier RJ**, **Regev A.** A single-cell survey of the small intestinal epithelium. *Nature* 551: 333–339, 2017. doi:10.1038/nature24489.
60. **Billipp TE**, **Fung C**, **Webeck LM**, **Sargent DB**, **Gologorsky MB**, **McDaniel MM**, **Kasal DN**, **McGinty JW**, **Barrow KA**, **Rich LM**, **Barilli A**, **Sabat M**, **Debley JS**, **Myers R**, **Howitt MR**, **von Moltke J.** Tuft cell-derived acetylcholine regulates epithelial fluid secretion (Preprint). *bioRxiv* 2023.03.17.533208, 2023. doi:10.1101/2023.03.17.533208.
61. **Schneider C**, **O'Leary CE**, **von Moltke J**, **Liang HE**, **Ang QY**, **Turnbaugh PJ**, **Radhakrishnan S**, **Pellizzon M**, **Ma A**, **Locksley RM.** A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. *Cell* 174: 271–284.e14, 2018. doi:10.1016/j.cell.2018.05.014.
62. **Ignacio A**, **Shah K**, **Bernier-Latmani J**, **Köller Y**, **Coakley G**, **Moyat M**, **Hamelin R**, **Armand F**, **Wong NC**, **Ramay H**, **Thomson CA**, **Burkhard R**, **Wang H**, **Dufour A**, **Geuking MB**, **McDonald B**, **Petrova TV**, **Harris NL**, **McCoy KD.** Small intestinal resident eosinophils maintain gut homeostasis following microbial colonization. *Immunity* 55: 1250–1267.e12, 2022. doi:10.1016/j.immuni.2022.05.014.
63. **Ye L**, **Bae M**, **Cassilly CD**, **Jabba SV**, **Thorpe DW**, **Martin AM**, **Lu HY**, **Wang J**, **Thompson JD**, **Lickwar CR**, **Poss KD**, **Keating DJ**, **Jordt SE**, **Clardy J**, **Liddle RA**, **Rawls JF.** Enteroendocrine cells sense bacterial tryptophan catabolites to activate enteric and vagal neuronal pathways. *Cell Host Microbe* 29: 179–196.e9, 2021. doi:10.1016/j.chom.2020.11.011.
64. **Sutherland TE**, **Dyer DP**, **Allen JE.** The extracellular matrix and the immune system: a mutually dependent relationship. *Science* 379: eabp8964, 2023. doi:10.1126/science.abp8964.
65. **Ning Y**, **Lin K**, **Fang J**, **Ding Y**, **Zhang Z**, **Chen X**, **Zhao Q**, **Wang H**, **Wang F.** Gastrointestinal pan-cancer landscape of tumor matrix heterogeneity identifies biologically distinct matrix stiffness subtypes predicting prognosis and chemotherapy efficacy. *Comput Struct Biotechnol J* 21: 2744–2758, 2023. doi:10.1016/j.csbj.2023.04.016.
66. **He S**, **Lei P**, **Kang W**, **Cheung P**, **Xu T**, **Mana M**, **Park CY**, **Wang H**, **Imada S**, **Russell JO**, **Wang J**, **Wang R**, **Zhou Z**, **Chetal K**, **Stas E**, **Mohad V**, **Bruun-Rasmussen P**, **Sadreyev RI**, **Hodin RA**, **Zhang Y**, **Breault DT**, **Camargo FD**, **Yilmaz ÖH**, **Fredberg JJ**, **Saeidi N.** Stiffness restricts the stemness of the intestinal stem cells and skews their differentiation toward goblet cells. *Gastroenterology* 164: 1137–1151.e15, 2023. doi:10.1053/j.gastro.2023.02.030.
67. **Wang Y**, **Kim R**, **Hinman SS**, **Zwarycz B**, **Magness ST**, **Allbritton NL.** Bioengineered systems and designer matrices that recapitulate the intestinal stem cell niche. *Cell Mol Gastroenterol Hepatol* 5: 440–453.e1, 2018. doi:10.1016/j.jcmgh.2018.01.008.
68. **Tardalkar K**, **Patil S**, **Chaudhari L**, **Kshersagar J**, **Damle M**, **Kawale A**, **Bhamare N**, **Desai V**, **Pathak N**, **Gaikwad V**, **Joshi MG.** Decellularized small intestine scaffolds: a potential xenograft for restoration of intestinal perforation. *Tissue Barriers* 2023: 2290940, 2023. doi:10.1080/21688370.2023.2290940.
69. **Wang F**, **Knutson K**, **Alcaino C**, **Linden DR**, **Gibbons SJ**, **Kashyap P**, **Grover M**, **Oeckler R**, **Gottlieb PA**, **Li HJ**, **Leiter AB**, **Farrugia G**, **Beyder A.** Mechanosensitive ion channel Piezo2 is important for enterochromaffin cell response to mechanical forces. *J Physiol* 595: 79–91, 2017. doi:10.1113/JP272718.
70. **Buchanan KL**, **Rupprecht LE**, **Kaelberer MM**, **Sahasrabudhe A**, **Klein ME**, **Villalobos JA**, **Liu WW**, **Yang A**, **Gelman J**, **Park S**, **Anikeeva P**, **Bohórquez DV.** The preference for sugar over sweetener depends on a gut sensor cell. *Nat Neurosci* 25: 191–200, 2022. doi:10.1038/s41593-021-00982-7.
71. **Bellono NW**, **Bayrer JR**, **Leitch DB**, **Castro J**, **Zhang C**, **O'Donnell TA**, **Brierley SM**, **Ingraham HA**, **Julius D.** enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell* 170: 185–198.16, 2017. doi:10.1016/j.cell.2017.05.034.
72. **Nadjsombati MS**, **McGinty JW**, **Lyons-Cohen MR**, **Jaffe JB**, **DiPeso L**, **Schneider C**, **Miller CN**, **Pollack JL**, **Nagana Gowda GA**, **Fontana MF**, **Erle DJ**, **Anderson MS**, **Locksley RM**, **Raftery D**, **von Moltke J.** Detection of succinate by intestinal tuft cells triggers a type 2 innate immune circuit. *Immunity* 49: 33–41.e7, 2018. doi:10.1016/j.immuni.2018.06.016.
73. **McGinty JW**, **Ting HA**, **Billipp TE**, **Nadjsombati MS**, **Khan DM**, **Barrett NA**, **Liang HE**, **Matsumoto I**, **von Moltke J.** Tuft-cell-derived leukotrienes drive rapid anti-helminth immunity in the small intestine but are dispensable for anti-protist immunity. *Immunity* 52: 528–541.e7, 2020. doi:10.1016/j.immuni.2020.02.005.
74. **Lai NY**, **Musser MA**, **Pinho-Ribeiro FA**, **Baral P**, **Jacobson A**, **Ma P**, **Potts DE**, **Chen Z**, **Paik D**, **Soualhi S**, **Yan Y**, **Misra A**, **Goldstein K**, **Lagomarsino VN**, **Nordstrom A**, **Sivanathan KN**, **Wallrapp A**, **Kuchroo VK**, **Nowarski R**, **Starnbach MN**, **Shi H**, **Surana NK**, **An D**, **Wu C**, **Huh JR**, **Rao M**, **Chiu IM.** Gut-innervating nociceptor neurons regulate Peyer's patch microfold cells and SFB levels to mediate *Salmonella* host defense. *Cell* 180: 33–49.e22, 2020. doi:10.1016/j.cell.2019.11.014.
75. **Yang D**, **Jacobson A**, **Meerschaert KA**, **Sifakis JJ**, **Wu M**, **Chen X**, **Yang T**, **Zhou Y**, **Anekal PV**, **Rucker RA**, **Sharma D**, **Sontheimer-Phelps A**, **Wu GS**, **Deng L**, **Anderson MD**, **Choi S**, **Neel D**, **Lee N**, **Kasper DL**, **Jabri B**, **Huh JR**, **Johansson M**, **Thiagarajah JR**, **Riesenfeld SJ**, **Chiu IM.** Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection. *Cell* 185: 4190–4205.e25, 2022. doi:10.1016/j.cell.2022.09.024.
76. **Gustafsson JK**, **Davis JE**, **Rappai T**, **McDonald KG**, **Kulkarni DH**, **Knoop KA**, **Hogan SP**, **Fitzpatrick JA**, **Lencer WI**, **Newberry RD.** Intestinal goblet cells sample and deliver lumenal antigens by regulated endocytic uptake and transcytosis. *eLife* 10: e67292, 2021. doi:10.7554/eLife.67292.
77. **Kulkarni DH**, **Gustafsson JK**, **Knoop KA**, **McDonald KG**, **Bidani SS**, **Davis JE**, **Floyd AN**, **Hogan SP**, **Hsieh CS**, **Newberry RD.** Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* 13: 271–282, 2020. doi:10.1038/s41385-019-0240-7.
78. **Talbot J**, **Littman DR.** Immune cell control of nutrient absorption. *Science* 371: 1202–1203, 2021. doi:10.1126/science.abg6455.
79. **Crifo B**, **MacNaughton WK.** Cells and mediators of inflammation as effectors of epithelial repair in the inflamed intestine. *Am J Physiol Gastrointest Liver Physiol* 322: G169–G182, 2022. doi:10.1152/ajpgi.00194.2021.
80. **Beck PL**, **Li Y**, **Wong J**, **Chen CW**, **Keenan CM**, **Sharkey KA**, **McCafferty DM.** Inducible nitric oxide synthase from bone marrow-derived cells plays a critical role in regulating colonic inflammation. *Gastroenterology* 132: 1778–1790, 2007. doi:10.1053/j.gastro.2007.01.032.
81. **MacEachern SJ**, **Patel BA**, **McKay DM**, **Sharkey KA.** Nitric oxide regulation of colonic epithelial ion transport: a novel role for enteric glia in the myenteric plexus. *J Physiol* 589: 3333–3348, 2011. doi:10.1113/jphysiol.2011.207902.
82. **MacEachern SJ**, **Patel BA**, **Keenan CM**, **Dicay M**, **Chapman K**, **McCafferty DM**, **Savidge TC**, **Beck PL**, **MacNaughton WK**, **Sharkey KA.** Inhibiting inducible nitric oxide synthase in enteric glia restores electrogenic ion transport in mice with colitis. *Gastroenterology* 149: 445–455.e3, 2015. doi:10.1053/j.gastro.2015.04.007.
83. **Brandt A**, **Baumann A**, **Hernández-Arriaga A**, **Jung F**, **Nier A**, **Staltner R**, **Rajcic D**, **Schmeer C**, **Witte OW**, **Wessner B**, **Franzke B**, **Wagner KH**, **Camarinha-Silva A**, **Bergheim I.** Impairments of intestinal arginine and NO metabolisms trigger aging-associated intestinal barrier dysfunction and 'inflammaging'. *Redox Biol* 58: 102528, 2022. doi:10.1016/j.redox.2022.102528.
84. **Abud HE**, **Amarasinghe SL**, **Micati D**, **Jardé T.** Stromal niche signals that orchestrate intestinal regeneration. *Cell Mol Gastroenterol Hepatol* 17: 679–685, 2024. doi:10.1016/j.jcmgh.2024.02.003.
85. **Petsakou A**, **Li Y**, **Liu Y**, **Comjean A**, **Hu Y**, **Perrimon N.** Cholinergic neurons trigger epithelial Ca^{2+} currents to heal the gut. *Nature* 623: 122–131, 2023. doi:10.1038/s41586-023-06627-y.
86. **Dhawan S**, **Hiemstra IH**, **Verseijden C**, **Hilbers FW**, **Te Velde AA**, **Willemsen LE**, **Stap J**, **den Haan JM**, **de Jonge WJ.** Cholinergic receptor activation on epithelia protects against cytokine-induced barrier dysfunction. *Acta Physiol (Oxf)* 213: 846–859, 2015 [Erratum in Acta Physiol (Oxf) 220: 501, 2017]. doi:10.1111/apha.12469.
87. **Costantini TW**, **Krzyzaniak M**, **Cheadle GA**, **Putnam JG**, **Hageny AM**, **Lopez N**, **Eliceiri BP**, **Bansal V**, **Coimbra R.** Targeting alpha-7 nicotinic acetylcholine receptor in the enteric nervous system: a cholinergic agonist prevents gut barrier failure after severe burn injury. *Am J Pathol* 181: 478–486, 2012. doi:10.1016/j.ajpath.2012.04.005.

Downloaded from journals.physiology.org/journal/ajpgi (172.232.036.207) on November 21, 2025.

88. **Brégeon J**, **Coron E**, **Da Silva AC**, **Jaulin J**, **Aubert P**, **Chevalier J**, **Vergnolle N**, **Meurette G**, **Neunlist M.** Sacral nerve stimulation enhances early intestinal mucosal repair following mucosal injury in a pig model. *J Physiol* 594: 4309–4323, 2016. doi:10.1113/JP271783.
89. **Drissi F**, **Bourreille A**, **Neunlist M**, **Meurette G.** Sacral neuromodulation for refractory ulcerative colitis: safety and efficacy in a prospective observational series of eight patients. *Tech Coloproctol* 27: 501–505, 2023. doi:10.1007/s10151-023-02793-3.
90. **Wang P**, **Kljavin N**, **Nguyen TT**, **Storm EE**, **Marsh B**, **Jiang J**, **Lin W**, **Menon H**, **Piskol R**, **de Sauvage FJ.** Adrenergic nerves regulate intestinal regeneration through IL-22 signaling from type 3 innate lymphoid cells. *Cell Stem Cell* 30: 1166–1178.e8, 2023. doi:10.1016/j.stem.2023.07.013.
91. **el Marjou F**, **Janssen KP**, **Chang BH**, **Li M**, **Hindie V**, **Chan L**, **Louvard D**, **Chambon P**, **Metzger D**, **Robine S.** Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* 39: 186–193, 2004. doi:10.1002/gene.20042.
92. **Peng Y**, **Huang Y**, **Li H**, **Li C**, **Wu Y**, **Wang X**, **Wang Q**, **He J**, **Miao C.** Associations between rheumatoid arthritis and intestinal flora, with special emphasis on RA pathologic mechanisms to treatment strategies. *Microb Pathog* 188: 106563, 2024. doi:10.1016/j.micpath.2024.106563.
93. **Wu S**, **Yang L**, **Fu Y**, **Liao Z**, **Cai D**, **Liu Z.** Intestinal barrier function and neurodegenerative disease. *CNS Neurol Disord Drug Targets* 23: 1134–1142, 2023. doi:10.2174/0118715273264097231116103948.
94. **Sato T**, **Vries RG**, **Snippert HJ**, **van de Wetering M**, **Barker N**, **Stange DE**, **van Es JH**, **Abo A**, **Kujala P**, **Peters PJ**, **Clevers H.** Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459: 262–265, 2009. doi:10.1038/nature07935.
95. **Rees WD**, **Telkar N**, **Lin DT**, **Wong MQ**, **Poloni C**, **Fathi A**, **Kobor M**, **Zachos NC**, **Steiner TS.** An in vitro chronic damage model impairs inflammatory and regenerative responses in human colonoid monolayers. *Cell Rep* 38: 110283, 2022. doi:10.1016/j.celrep.2021.110283.
96. **Gómez DP**, **Boudreau F.** Organoids and their use in modeling gut epithelial cell lineage differentiation and barrier properties during intestinal diseases. *Front Cell Dev Biol* 9: 732137, 2021. doi:10.3389/fcell.2021.732137.
97. **Margiotta F**, **Di Cesare Mannelli L**, **Morabito A**, **Ghelardini C**, **Lucarini E.** Investigating epithelial-neuronal signaling contribution in visceral pain through colon organoid-dorsal root ganglion neuron co-cultures. *Neural Regen Res* 19: 1199–1200, 2024. doi:10.4103/1673-5374.386403.
98. **Kechele DO**, **Wells JM.** Recent advances in deriving human endodermal tissues from pluripotent stem cells. *Curr Opin Cell Biol* 61: 92–100, 2019. doi:10.1016/j.ceb.2019.07.009.
99. **Workman MJ**, **Mahe MM**, **Trisno S**, **Poling HM**, **Watson CL**, **Sundaram N**, **Chang CF**, **Schiesser J**, **Aubert P**, **Stanley EG**, **Elefanty AG**, **Miyaoka Y**, **Mandegar MA**, **Conklin BR**, **Neunlist M**, **Brugmann SA**, **Helmrath MA**, **Wells JM.** Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. *Nat Med* 23: 49–59, 2017. doi:10.1038/nm.4233.
100. **Ayyaz A**, **Kumar S**, **Sangiorgi B**, **Ghoshal B**, **Gosio J**, **Ouladan S**, **Fink M**, **Barutcu S**, **Trcka D**, **Shen J**, **Chan K**, **Wrana JL**, **Gregorieff A.** Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. *Nature* 569: 121–125, 2019. doi:10.1038/s41586-019-1154-y.
101. **Duan S**, **Sawyer TW**, **Witten BL**, **Song H**, **Else T**, **Merchant JL.** Spatial profiling reveals tissue-specific neuro-immune interactions in gastroenteropancreatic neuroendocrine tumors. *J Pathol* 262: 362–376, 2024. doi:10.1002/path.6241.
102. **Grill M**, **Hasenoehrl C**, **Kienzl M**, **Kargl J**, **Schicho R.** Cellular localization and regulation of receptors and enzymes of the endocannabinoid system in intestinal and systemic inflammation. *Histochem Cell Biol* 151: 5–20, 2019. doi:10.1007/s00418-018-1719-0.
103. **Ferrian S**, **Liu CC**, **McCaffrey EF**, **Kumar R**, **Nowicki TS**, **Dawson DW**, **Baranski A**, **Glaspy JA**, **Ribas A**, **Bendall SC**, **Angelo M.** Multiplexed imaging reveals an IFN-gamma-driven inflammatory state in nivolumab-associated gastritis. *Cell Rep Med* 2: 100419, 2021. doi:10.1016/j.xcrm.2021.100419.
104. **Zeisel A**, **Hochgerner H**, **Lönnerberg P**, **Johnsson A**, **Memic F**, **van der Zwan J**, **Häring M**, **Braun E**, **Borm LE**, **La Manno G**, **Codeluppi S**, **Furlan A**, **Lee K**, **Skene N**, **Harris KD**, **Hjerling-Leffler J**, **Arenas E**, **Ernfors P**, **Marklund U**, **Linnarsson S.** Molecular architecture of the mouse nervous system. *Cell* 174: 999–1014.e1022, 2018. doi:10.1016/j.cell.2018.06.021.
105. **Wright CM**, **Schneider S**, **Smith-Edwards KM**, **Mafra F**, **Leembruggen AJ**, **Gonzalez MV**, **Kothakapa DR**, **Anderson JB**, **Maguire BA**, **Gao T**, **Missall TA**, **Howard MJ**, **Bornstein JC**, **Davis BM**, **Heuckeroth RO.** scRNA-Seq reveals new enteric nervous system roles for GDNF, NRTN, and TBX3. *Cell Mol Gastroenterol Hepatol* 11: 1548–1592.e1, 2021. doi:10.1016/j.jcmgh.2020.12.014.
106. **Morarach K**, **Mikhailova A**, **Knoflach V**, **Memic F**, **Kumar R**, **Li W**, **Ernfors P**, **Marklund U.** Diversification of molecularly defined myenteric neuron classes revealed by single-cell RNA sequencing. *Nat Neurosci* 24: 34–46, 2021. doi:10.1038/s41593-020-00736-x.
107. **Drokhlyansky E**, **Smillie CS**, **Van Wittenberghe N**, **Ericsson M**, **Griffin GK**, **Eraslan G**, **Dionne D**, **Cuoco MS**, **Goder-Reiser MN**, **Sharova T**, **Kuksenko O**, **Aguirre AJ**, **Boland GM**, **Graham D**, **Rozenblatt-Rosen O**, **Xavier RJ**, **Regev A.** The human and mouse enteric nervous system at single-cell resolution. *Cell* 182: 1606–1622.e23, 2020. doi:10.1016/j.cell.2020.08.003.
108. **May-Zhang AA**, **Tycksen E**, **Southard-Smith AN**, **Deal KK**, **Benthal JT**, **Buehler DP**, **Adam M**, **Simmons AJ**, **Monaghan JR**, **Matlock BK**, **Flaherty DK**, **Potter SS**, **Lau KS**, **Southard-Smith EM.** Combinatorial transcriptional profiling of mouse and human enteric neurons identifies shared and disparate subtypes in situ. *Gastroenterology* 160: 755–770.e26, 2021. doi:10.1053/j.gastro.2020.09.032.
109. **Majd A**, **Richter MN**, **Samuel RM**, **Cesiulis A**, **Ghazizadeh Z**, **Wang J**, **Fattahi F.** Combined GWAS and single cell transcriptomics uncover the underlying genes and cell types in disorders of gut-brain interaction (Preprint). *medRxiv* 2023.06.02.23290906, 2023. doi:10.1101/2023.06.02.23290906.
110. **Rao A**, **Barkley D**, **França GS**, **Yanai I.** Exploring tissue architecture using spatial transcriptomics. *Nature* 596: 211–220, 2021. doi:10.1038/s41586-021-03634-9.
111. **Ståhl PL**, **Salmén F**, **Vickovic S**, **Lundmark A**, **Navarro JF**, **Magnusson J**, **Giacomello S**, **Asp M**, **Westholm JO**, **Huss M**, **Mollbrink A**, **Linnarsson S**, **Codeluppi S**, **Borg A**, **Pontén F**, **Costea PI**, **Sahlén P**, **Mulder J**, **Bergmann O**, **Lundeberg J**, **Frisén J.** Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 353: 78–82, 2016. doi:10.1126/science.aaf2403.
112. **Wolf J**, **Rasmussen DK**, **Sun YJ**, **Vu JT**, **Wang E**, **Espinosa C**, **Bigini F**, **Chang RT**, **Montague AA**, **Tang PH**, **Mruthyunjaya P**, **Aghaeepour N**, **Dufour A**, **Bassuk AG**, **Mahajan VB.** Liquid-biopsy proteomics combined with AI identifies cellular drivers of eye aging and disease in vivo. *Cell* 186: 4868–4884.e12, 2023. doi:10.1016/j.cell.2023.09.012.
113. **Cavin JB**, **Wongkrasant P**, **Glover JB**, **Balemba OB**, **MacNaughton WK**, **Sharkey KA.** Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice. *J Physiol* 601: 1183–1206, 2023. doi:10.1113/JP284171.
114. **Barth BB**, **Redington ER**, **Gautam N**, **Pelot NA**, **Grill WM.** Calcium image analysis in the moving gut. *Neurogastroenterol Motil* 35: e14678, 2023. doi:10.1111/nmo.14678.
115. **Drumm BT**, **Cobine CA**, **Baker SA.** Insights on gastrointestinal motility through the use of optogenetic sensors and actuators. *J Physiol* 600: 3031–3052, 2022. doi:10.1113/JP281930.
116. **Spencer NJ**, **Hibberd T**, **Feng J**, **Hu H.** Optogenetic control of the enteric nervous system and gastrointestinal transit. *Expert Rev Gastroenterol Hepatol* 13: 281–284, 2019. doi:10.1080/17474124.2019.1581061.
117. **Smith-Edwards KM**, **Edwards BS**, **Wright CM**, **Schneider S**, **Meerschaert KA**, **Ejoh LL**, **Najjar SA**, **Howard MJ**, **Albers KM**, **Heuckeroth RO**, **Davis BM.** Sympathetic input to multiple cell types in mouse and human colon produces region-specific responses. *Gastroenterology* 160: 1208–1223.e4, 2021. doi:10.1053/j.gastro.2020.09.030.
118. **Nestor-Kalinoski A**, **Smith-Edwards KM**, **Meerschaert K**, **Margiotta JF**, **Rajwa B**, **Davis BM**, **Howard MJ.** Unique neural circuit connectivity of mouse proximal, middle, and distal colon defines regional colonic motor patterns. *Cell Mol Gastroenterol Hepatol* 13: 309–337.e3, 2022. doi:10.1016/j.jcmgh.2021.08.016.
119. **Bayrer JR**, **Castro J**, **Venkataraman A**, **Touhara KK**, **Rossen ND**, **Morrie RD**, **Maddern J**, **Hendry A**, **Braverman KN**, **Garcia-Caraballo S**, **Schober G**, **Brizuela M**, **Castro Navarro FM**, **Bueno-Silva C**, **Ingraham HA**, **Brierley SM**, **Julius D.** Gut enterochromaffin cells

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drive visceral pain and anxiety. *Nature* 616: 137–142, 2023. doi:10.1038/s41586-023-05829-8.

120. **Treichel AJ**, **Finholm I**, **Knutson KR**, **Alcaino C**, **Whiteman ST**, **Brown MR**, **Matveyenko A**, **Wegner A**, **Kacmaz H**, **Mercado-Perez A**, **Gajdos GB**, **Ordog T**, **Grover M**, **Szurszewski J**, **Linden DR**, **Farrugia G**, **Beyder A.** Specialized mechanosensory epithelial cells in mouse gut intrinsic tactile sensitivity. *Gastroenterology* 162: 535–547.e13, 2022. doi:10.1053/j.gastro.2021.10.026.
121. **Najjar SA**, **Edwards BS**, **Albers KM**, **Davis BM**, **Smith-Edwards KM.** Optogenetic activation of the distal colon epithelium engages enteric nervous system circuits to initiate motility patterns. *Am J Physiol Gastrointest Liver Physiol* 321: G426–G435, 2021. doi:10.1152/ajpgi.00026.2021.
122. **Leven P**, **Schneider R**, **Schneider L**, **Mallesh S**, **Vanden Berghe P**, **Sasse P**, **Kalff JC**, **Wehner S.** beta-adrenergic signaling triggers enteric glial reactivity and acute enteric gliosis during surgery. *J Neuroinflammation* 20: 255, 2023. doi:10.1186/s12974-023-02937-0.
123. **Scavuzzo MA**, **Letai KC**, **Maeno-Hikichi Y**, **Wulftange WJ**, **Shah IK**, **Rameshbabu JS**, **Tomar A**, **Shick HE**, **Shah AK**, **Xiong Y**, **Cohn EF**, **Allan KC**, **Tesar PJ.** Enteric glial hub cells coordinate intestinal motility (Preprint). *bioRxiv* 2023.06.07.544052, 2023. doi:10.1101/2023.06.07.544052.
124. **Baghdadi MB**, **Ayyaz A**, **Coquenlorge S**, **Chu B**, **Kumar S**, **Streutker C**, **Wrana JL**, **Kim TH.** Enteric glial cell heterogeneity regulates intestinal stem cell niches. *Cell Stem Cell* 29: 86–100.e106, 2022. doi:10.1016/j.stem.2021.10.004.
125. **Ahmadzai MM**, **Seguella L**, **Gulbransen BD.** Circuit-specific enteric glia regulate intestinal motor neurocircuits. *Proc Natl Acad Sci USA* 118: e2025938118, 2021. doi:10.1073/pnas.2025938118.
126. **Schneider KM**, **Blank N**, **Alvarez Y**, **Thum K**, **Lundgren P**, **Litichevskiy L**, **et al.** The enteric nervous system relays psychological stress to intestinal inflammation. *Cell* 186: 2823–2838.e20, 2023. doi:10.1016/j.cell.2023.05.001.
127. **Grundy L**, **Erickson A**, **Brierley SM.** Visceral Pain. *Annu Rev Physiol* 81: 261–284, 2019. doi:10.1146/annurev-physiol-020518-114525.
128. **Browning KN**, **Verheijden S**, **Boeckxstaens GE.** The vagus nerve in appetite regulation, mood, and intestinal inflammation. *Gastroenterology* 152: 730–744, 2017. doi:10.1053/j.gastro.2016.10.046.
129. **Brookes S**, **Chen N**, **Humenick A**, **Spencer NJ**, **Costa M.** Extrinsic sensory innervation of the gut: structure and function. *Adv Exp Med Biol* 891: 63–69, 2016. doi:10.1007/978-3-319-27592-5_7.
130. **Phillips RJ**, **Powley TL.** Innervation of the gastrointestinal tract: patterns of aging. *Auton Neurosci* 136: 1–19, 2007. doi:10.1016/j.autneu.2007.04.005.
131. **Yu S**, **Undem BJ**, **Kollarik M.** Vagal afferent nerves with nociceptive properties in guinea-pig oesophagus. *J Physiol* 563: 831–842, 2005. doi:10.1113/jphysiol.2004.079574.
132. **Powley TL.** Brain-gut communication: vagovagal reflexes interconnect the two "brains". *Am J Physiol Gastrointest Liver Physiol* 321: G576–G587, 2021. doi:10.1152/ajpgi.00214.2021.
133. **Forsythe P**, **Bienenstock J**, **Kunze WA.** Vagal pathways for microbiome-brain-gut axis communication. *Adv Exp Med Biol* 817: 115–133, 2014. doi:10.1007/978-1-4939-0897-4_5.
134. **Goehler LE**, **Gaykema RP**, **Hansen MK**, **Anderson K**, **Maier SF**, **Watkins LR.** Vagal immune-to-brain communication: a visceral chemosensory pathway. *Auton Neurosci* 85: 49–59, 2000. doi:10.1016/S1566-0702(00)00219-8.
135. **Abdullah N**, **Defaye M**, **Altier C.** Neural control of gut homeostasis. *Am J Physiol Gastrointest Liver Physiol* 319: G718–G732, 2020. doi:10.1152/ajpgi.00293.2020.
136. **Grubišić V**, **McClain JL**, **Fried DE**, **Grants I**, **Rajasekhar P**, **Csizmadia E**, **Ajijola OA**, **Watson RE**, **Poole DP**, **Robson SC**, **Christofi FL**, **Gulbransen BD.** Enteric glia modulate macrophage phenotype and visceral sensitivity following inflammation. *Cell Rep* 32: 108100, 2020. doi:10.1016/j.celrep.2020.108100.
137. **Morales-Soto W**, **Gonzales J**, **Jackson WF**, **Gulbransen BD.** Enteric glia promote visceral hypersensitivity during inflammation through intercellular signaling with gut nociceptors. *Sci Signal* 16: eadg1668, 2023. doi:10.1126/scisignal.adg1668.
138. **Basso L**, **Garnier L**, **Bessac A**, **Boué J**, **Blanpied C**, **Cenac N**, **Laffont S**, **Dietrich G.** T-lymphocyte-derived enkephalins reduce T_h1/T_h17 colitis and associated pain in mice. *J Gastroenterol* 53: 215–226, 2018. doi:10.1007/s00535-017-1341-2.
139. **Cuddihey H**, **MacNaughton WK**, **Sharkey KA.** Role of the endocannabinoid system in the regulation of intestinal homeostasis. *Cell Mol Gastroenterol Hepatol* 14: 947–963, 2022. doi:10.1016/j.jcmgh.2022.05.015.
140. **Aguilera-Lizarraga J**, **Florens MV**, **Viola MF**, **Jain P**, **Decraecker L**, **Appeltans I**, **et al.** Local immune response to food antigens drives meal-induced abdominal pain. *Nature* 590: 151–156, 2021. doi:10.1038/s41586-020-03118-2.
141. **Verdú EF**, **Bercik P**, **Verma-Gandhu M**, **Huang XX**, **Blennerhassett P**, **Jackson W**, **Mao Y**, **Wang L**, **Rochat F**, **Collins SM.** Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 55: 182–190, 2006. doi:10.1136/gut.2005.066100.
142. **Esquerre N**, **Basso L**, **Defaye M**, **Vicentini FA**, **Cluny N**, **Bihan D**, **Hirota SA**, **Schick A**, **Jijon HB**, **Lewis IA**, **Geuking MB**, **Sharkey KA**, **Altier C**, **Nasser Y.** Colitis-induced microbial perturbation promotes postinflammatory visceral hypersensitivity. *Cell Mol Gastroenterol Hepatol* 10: 225–244, 2020. doi:10.1016/j.jcmgh.2020.04.003.
143. **Chiu IM**, **Heesters BA**, **Ghasemlou N**, **Von Hehn CA**, **Zhao F**, **Tran J**, **Wainger B**, **Strominger A**, **Muralidharan S**, **Horswill AR**, **Bubeck Wardenburg J**, **Hwang SW**, **Carroll MC**, **Woolf CJ.** Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* 501: 52–57, 2013. doi:10.1038/nature12479.
144. **Shin A**, **Preidis GA**, **Shulman R**, **Kashyap PC.** The gut microbiome in adult and pediatric functional gastrointestinal disorders. *Clin Gastroenterol Hepatol* 17: 256–274, 2019. doi:10.1016/j.cgh.2018.08.054.
145. **McIntosh K**, **Reed DE**, **Schneider T**, **Dang F**, **Keshteli AH**, **De Palma G**, **Madsen K**, **Bercik P**, **Vanner S.** FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut* 66: 1241–1251, 2017. doi:10.1136/gutjnl-2015-311339.
146. **De Palma G**, **Shimbori C**, **Reed DE**, **Yu Y**, **Rabbia V**, **Lu J**, **Jimenez-Vargas N**, **Sessenwein J**, **Lopez-Lopez C**, **Pigrau M**, **Jaramillo-Polanco J**, **Zhang Y**, **Baerg L**, **Manzar A**, **Pujo J**, **Bai X**, **Pinto-Sanchez MI**, **Caminero A**, **Madsen K**, **Surette MG**, **Beyak M**, **Lomax AE**, **Verdu EF**, **Collins SM**, **Vanner SJ**, **Bercik P.** Histamine production by the gut microbiota induces visceral hyperalgesia through histamine 4 receptor signaling in mice. *Sci Transl Med* 14: eabj1895, 2022. doi:10.1126/scitranslmed.abj1895.
147. **Keogh CE**, **Kim DH**, **Pusceddu MM**, **Knotts TA**, **Rabasa G**, **Sladek JA**, **Hsieh MT**, **Honeycutt M**, **Brust-Mascher I**, **Barboza M**, **Gareau MG.** Myelin as a regulator of development of the microbiota-gut-brain axis. *Brain Behav Immun* 91: 437–450, 2021. doi:10.1016/j.bbi.2020.11.001.
148. **Hennessey C**, **Keogh CE**, **Barboza M**, **Brust-Mascher I**, **Knotts TA**, **Sladek JA**, **Pusceddu MM**, **Stokes P**, **Rabasa G**, **Honeycutt M**, **Walsh O**, **Nichols R**, **Reardon C**, **Gareau MG.** Neonatal enteropathogenic *Escherichia coli* infection disrupts microbiota-gut-brain axis signaling. *Infect Immun* 89: e0005921, 2021. doi:10.1128/IAI.00059-21.
149. **Morton JT**, **Jin DM**, **Mills RH**, **Shao Y**, **Rahman G**, **McDonald D**, **et al.** Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. *Nat Neurosci* 26: 1208–1217, 2023. doi:10.1038/s41593-023-01361-0.
150. **Gracie DJ**, **Williams CJ**, **Sood R**, **Mumtaz S**, **Bholah MH**, **Hamlin PJ**, **Ford AC.** Negative effects on psychological health and quality of life of genuine irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 15: 376–384.e5, 2017. doi:10.1016/j.cgh.2016.05.012.
151. **Janssen LM**, **Rezazadeh Ardabili A**, **Romberg-Camps MJ**, **Winkens B**, **van den Broek RJ**, **Hulst J**, **Verwijs HJ**, **Keszthelyi D**, **Jonkers D**, **van Bodegraven AA**, **Pierik MJ**, **Mujagic Z.** Abdominal pain in patients with inflammatory bowel disease in remission: a prospective study on contributing factors. *Aliment Pharmacol Ther* 58: 1041–1051, 2023. doi:10.1111/apt.17718.
152. **Oka P**, **Parr H**, **Barberio B**, **Black CJ**, **Savarino EV**, **Ford AC.** Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 5: 908–917, 2020. doi:10.1016/S2468-1253(20)30217-X.
153. **Tramullas M**, **Collins JM**, **Fitzgerald P**, **Dinan TG**, **O'Mahony SM**, **Cryan JF.** Estrous cycle and ovariectomy-induced changes in visceral pain are microbiota-dependent. *iScience* 24: 102850, 2021. doi:10.1016/j.isci.2021.102850.
154. **Pujo J**, **De Palma G**, **Lu J**, **Galipeau HJ**, **Surette MG**, **Collins SM**, **Bercik P.** Gut microbiota modulates visceral sensitivity through calcitonin gene-related peptide (CGRP) production. *Gut Microbes* 15: 2188874, 2023. doi:10.1080/19490976.2023.2188874.

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155. **Armbruster BN**, **Li X**, **Pausch MH**, **Herlitze S**, **Roth BL.** Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc Natl Acad Sci USA* 104: 5163–5168, 2007. doi:10.1073/pnas.0700293104.
156. **Boyden ES**, **Zhang F**, **Bamberg E**, **Nagel G**, **Deisseroth K.** Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 8: 1263–1268, 2005. doi:10.1038/nn1525.
157. **Deisseroth K.** Optogenetics: 10 years of microbial opsins in neuroscience. *Nat Neurosci* 18: 1213–1225, 2015. doi:10.1038/nn.4091.
158. **Sternson SM**, **Roth BL.** Chemogenetic tools to interrogate brain functions. *Annu Rev Neurosci* 37: 387–407, 2014. doi:10.1146/annurev-neuro-071013-014048.
159. **Defaye M**, **Abdullah NS**, **Iftinca M**, **Hassan A**, **Agosti F**, **Zhang Z**, **Cumenal M**, **Zamponi GW**, **Altier C.** Gut-innervating TRPV1+ neurons drive chronic visceral pain via microglial P2Y12 receptor. *Cell Mol Gastroenterol Hepatol* 13: 977–999, 2022. doi:10.1016/j.jcmgh.2021.12.012.
160. **Agulhon C**, **Boyt KM**, **Xie AX**, **Friocourt F**, **Roth BL**, **McCarthy KD.** Modulation of the autonomic nervous system and behaviour by acute glial cell Gq protein-coupled receptor activation in vivo. *J Physiol* 591: 5599–5609, 2013. doi:10.1113/jphysiol.2013.261289.
161. **Tamari M**, **Del Bel KL**, **Ver Heul AM**, **Zamidar L**, **Orimo K**, **Hoshi M**, **et al.** Sensory neurons promote immune homeostasis in the lung. *Cell* 187: 44–61.e17, 2024. doi:10.1016/j.cell.2023.11.027.

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